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MEDICAL BIOCHEMISTRY



SECOND EDITION • COMPLETELY REVISED



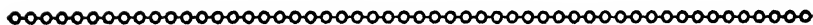
MEDICAL BIOCHEMISTRY

By

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WITH 106 TABLES

HAMISH HAMILTON MEDICAL BOOKS

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MEDICAL BIOCHEMISTRY

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SECOND EDITION, COMPLETELY REVISED, 1946

FIRST EDITION, 1942

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To
ALICE

PREFACE TO SECOND EDITION

A critical revision of the text was undertaken to provide accurate modern information for students and others who regard the book as a convenient reference volume. Certain topics have been expanded, especially the following: anoxia, carboxylation and decarboxylation, the chemistry of development, hemoglobinuria, hypertensive factors, ketogenesis, the metabolism of bile acids, muscle diseases, oxidizing-reducing enzyme systems, peptidases, phosphatases, plasma proteins, renal function, spreading factor, steride hormones, sulfonamides, transamination, transmethylation, vitamins A and K, and vitamins of the B complex. New subjects include adaptive enzymes, amino acid therapy, antibiotic substances, cephalin fractions, cirrhosis, dicumarol, gangliosides, phosphorylation, the Rh factor, and thiouracil. Careful attention has been given to selection of data and references. The system of cross references has been improved, and a new convenient index prepared. Certain tables have been extensively revised; new tabulations include antibiotic substances, bacterial polysaccharide haptens, and turnover numbers of enzymes.

The usefulness of a scientific book depends largely on the extent and quality of the information which it provides, and these criteria are qualified by the author's acquaintance with the pertinent literature. Attainment of perfection in the task is an ever receding goal, due to an expanding literature. The discomfort of prolonged sessions at the typewriter or in crowded library stacks, with the temperature hovering around a hundred degrees, is less discouraging than the realization that numerous fine publications cannot be examined at leisure. In this dilemma, suggestions from associates and interested readers are very helpful. Numerous improvements in the present edition originated from correspondence, and from discussions in clerkship and intern conferences.

The author is especially indebted to the following associates for suggestions, criticisms, and technical aid: Louis E. Diamond, Prof. Arthur A. Hellbaum, Prof. Howard C. Hopps, Prof. Alton C. Kurtz, Prof. Homer P. Marsh, Miss Fay Sheppard, Miss Gladys Jones, and the editor and publishers.

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PREFACE TO FIRST EDITION

In this text the author has attempted a modern, concise and correlated survey of biochemical knowledge for students of medicine and allied subjects. The selection and arrangement of material follow the suggestions of Dr. Fred C. Zapffe, secretary of the Association of American Medical Colleges, to devise "a text in consonance with modern teaching, which includes the essential facts and data fundamental to a basic medical education, eliminates discussion of pros and cons, and is of such size that the student will have time to read it."

The author has endeavored to assist students in their search for truth by avoiding unnecessary speculations and argumentative considerations. The semi-historical, speculative method of presentation, commonly used in older biochemistry texts, has been replaced by the straight-forward, systematic outline of fundamentals found acceptable by the more highly developed sciences. The organization of the subject matter has been guided by the criteria of simple logical presentation, thorough discussion of each topic as a unit, and tabulation of details. This method has facilitated the inclusion of considerable fundamental biochemical data in an abbreviated volume.

Each chapter is divided into separate sections which deal with chemistry, metabolism, and pathology, respectively, an arrangement which is suited to various types of instruction. The chemical and metabolic sections are carefully organized for study by the beginner and for rapid review by the advanced student; the pathology sections contain systematic discussions of the biochemical aspects of diagnosis and therapy. Correlations of biochemistry to other medical subjects are stressed; and bibliographies of selected modern reviews are given.

The subject matter includes certain topics not found in contemporary texts, as, for example, discussions of logarithms, the vehicular functions of plasma proteins, permeability of capillaries and placenta, biliary and renal calculi, colloid chemistry in histology, bacterial activities in the intestine, principles of diet therapy, the lipidoses, metabolism of acids related to carbohydrates, the chemistry of immunity and heredity, metabolism of bone, the phosphatases, and the metabolic interrelations of minerals.

Important discoveries of recent date have been systematically correlated with older knowledge. Modern views are featured especially in the discussions of blood clotting, experiments with isotopes, chemistry of the

carotenoids, carcinogenic and caryotoxic substances, growth factors, lipotropic factors, ketogenesis, glycogenesis, acetylcholine metabolism, the cholinergic diseases, fixation of carbon, biological methylation, transamination and transamidation, protein structure and molecular weights, association and denaturation of proteins, the chemistry of antigens, antibodies, haptens, virus nucleoproteins and inductors, also of hypertensive factors, porphyrias, and the chemistry of porphyrins.

The summarizing chapters on vitamins and hormones, which are based largely upon recent researches, and the sections on phospholipide, steride, protein, and mineral metabolism, have been presented in particularly systematic fashion.

The author is deeply indebted to the following friends for active assistance in the project: the editor and publishers; Miss Fay Sheppard, Mr. Louis E. Diamond, Prof. Irvin S. Danielson and Prof. Arthur Hellbaum of the University of Oklahoma School of Medicine; also to his wife, Alice, for sympathetic encouragement and patient assistance, and to numerous professional colleagues, physicians and medical students for suggestions and advice.

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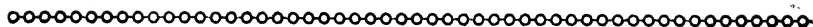
MEDICAL BIOCHEMISTRY



"The historical introduction is very much like the chaplain's prayer which opens a legislative session or political convention, very little of the subsequent proceedings is decided by reference to it." — MORRIS R. COHEN

CHAPTER I

ACID-BASE RELATIONS

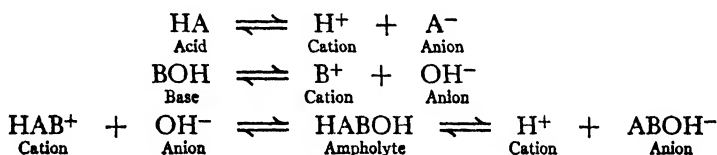


CHEMISTRY

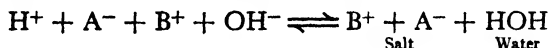
"As a reaction-system, the organism remains in continuous equilibrium so long as the relevant factors in the environment remain the same." — MORRIS R. COHEN

ELECTROLYTIC DISSOCIATION

Electrolytes in aqueous solution dissociate more or less completely into positively charged cations and negatively charged anions. This dissociation has been shown by the effects of ions on osmotic pressure, conductivity, and lowering of the freezing point. In aqueous solutions, weak acids or bases are only partially dissociated into hydrogen ions (protons) and anions, or cations and hydroxyl ions, respectively. Substances which can dissociate either as acid or base are termed *ampholytes*.



The reaction between acid and base in aqueous solution is as follows:



This neutralization reaction proceeds practically quantitatively to the right, since water ionizes to a very slight extent. The equilibrium which results from partial dissociation of a weak acid or base is an important concept to which we shall return.

The amount of base required to neutralize a certain volume of acid, or vice versa, is called the *titratable acidity* or *alkalinity*; these values are customarily expressed in terms of normality. A *normal solution* is one which contains per liter one gram atomic weight of replaceable hydrogen or its equivalent. This should be distinguished from a *molar solution* which contains per liter one gram molecular weight, or mol, of the com-

pound. For example, in the case of sulfuric acid, with molecular weight of 98.08 and two replaceable or ionizable hydrogens, the molar solution contains 98.08 gm. per liter, while the normal solution contains only 49.04 gm. per liter. In the special case of monovalent acids and bases, a given volume of the normal solution contains the same mass of hydrogen or hydroxyl ion as the molar solution.

MANIFESTATIONS OF ACIDITY AND ALKALINITY

The total number of potential hydrogen or hydroxyl ions is determined by titration, and is expressed as milliliters of normal acid or base. Titration thus measures the total available hydrogen or hydroxyl in the acid or base used, but it tells nothing of the degree of ionization of the acid or base. It is important to differentiate clearly between these two manifestations; namely, the total replaceable or potentially ionizable hydrogen called the titratable acidity; and the actual extent of the ionization of the hydrogen in any particular solution, expressible as pH. The first represents the quantity of acid available; the second measures the tendency of the acid to affect chemical systems sensitive to hydrogen ions. It is necessary to distinguish, in the same manner, between the total replaceable hydroxyl ion and the extent of the ionization into hydroxyl ion, expressible as pOH. The effects of acids and bases in living organisms and biological material are closely related to the actual concentrations of hydrogen and hydroxyl ions, that is, to the pH and pOH.

MASS LAW AND IONIZATION CONSTANTS

To obtain a clear conception of the term pH, it is necessary to study the chemical equilibrium in an acid solution. Equilibrium is a state of balance between opposing forces which are, apparently, but not actually, at rest. In a closed bottle partly filled with water, a constant number of water molecules exist as vapor in the air space as long as the temperature and pressure are constant. Although constant in number, they are not the same molecules. Two opposing processes are occurring, namely, the escape of molecules from the liquid phase into the gaseous phase and vice versa. The system is in a state of kinetic equilibrium when equal numbers of water molecules enter and leave the liquid phase in a given unit of time.

The similar kinetic chemical equilibria in dilute solutions are governed by the *law of mass action*, which states that the velocity of a chemical reaction is proportional to the concentrations of the reacting substances. When two substances, A and B, react in dilute solution to form C and D, the reaction is subject to two opposing influences. At first the reaction, $A + B \longrightarrow C + D$, is rapid, for neither C nor D is present; but as soon as C and D are formed the opposing reaction, $C + D \longrightarrow A + B$, takes place. As the concentrations of C and D increase in solution, the rate of

formation of A and B also increases. Eventually the two reactions reach an equilibrium with fixed concentrations of all four constituents. At equilibrium, the concentrations of A, B, C, and D may differ, but the velocities of the two opposing reactions are equal. The equation for the equilibrium is as follows: $A + B \rightleftharpoons C + D$. At equilibrium, there is an important relation between the concentrations of the reacting substances. The product of the molar concentrations of the reacting substances on one side of the equation has a constant relation to the product of the reacting substances on the other side. At any given temperature this relationship, known as the *mass law*, is conventionally expressed as

$$\frac{[C][D]}{[A][B]} = K$$

where K is the *equilibrium constant* for the reaction. Brackets are used to express the molar concentrations. The detailed derivation of this equation may be found in general chemistry texts.¹

The ionization of acids, a reversible chemical reaction, is subject to the mass law.² For example, acetic acid ionizes as follows: $\text{CH}_3\text{COOH} \rightleftharpoons \text{H}^+ + \text{CH}_3\text{COO}^-$. According to the mass law,

$$\frac{[\text{H}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} = K$$

the ionization constant of acetic acid. An acid with more than one replaceable hydrogen will have as many constants as replaceable hydrogens, thus: $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$, and $\text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{--}$;

or,
$$\frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = K_1$$

and
$$\frac{[\text{H}^+][\text{CO}_3^{--}]}{[\text{HCO}_3^-]} = K_2$$

In calculating the $[\text{H}^+]$ of solutions of dibasic acids, the second ionization can usually be neglected. In Table 1, the approximate ionization constants of many acids and bases, of biochemical interest, are given as pK , which is defined as

$$\log \frac{1}{K}$$

¹ There is no relation between the rate at which a reaction reaches equilibrium and the magnitude of its reaction constant. The velocity of a reaction is increased by (1) temperature (approximately twofold for every 10° C. increase in temperature), and (2) the presence of certain substances which have specific effects (called positive catalysts).

² Since strong acids (or bases), such as hydrochloric acid and sulfuric acid, are ionized highly in aqueous solutions, the ion concentrations are approximately equal to the normality of the solution. In a 0.1 N hydrochloric acid solution $[\text{H}^+] = [\text{Cl}^-] = 1 \times 10^{-1}$.

or the negative logarithm of the dissociation constant. The pK values are affected by electrolyte concentration.

TABLE 1
APPROXIMATE DISSOCIATION CONSTANTS¹
(As Negative Logarithms)

	pK_{acid} (pK_H)				pK_{base} (pK_{OH})		
	1	2	3	4	1	2	3
Acetamide					14.5		
Acetic acid	4.7						
Acetoacetic acid	3.6						
Adenine					4.1		
Adenosine					3.5		
Adenylic acid, muscle		6.2			3.8		
Adenylic acid, yeast	0.9	6.0			3.7		
Alanine	2.3				9.7		
β -Alanine	3.6						
Alanylanine	3.2				8.4		
Alanylglycine	3.1				8.2		
Alanylproline	3.0				8.4		
Aluminum hydroxide					12.2		
<i>p</i> -Aminobenzoic acid	2.4				4.8		
Ammonium hydroxide					4.7		
Anserine	2.6				7.0	9.5	
Arginine	2.2				9.0	12.5	
Arsenic acid	2.3	4.4	9.2				
Ascorbic acid	4.2	11.6					
Asparagine	2.1				8.9		
Aspartic acid	1.9	3.7			9.6		
Aspartylaspartic acid	2.7	3.4	4.7		8.3		
Aspartylglycine	2.1	4.5			9.1		
α -Aspartylhistidine	2.5	3.0			6.8	8.0	
Aspartyltyrosine	2.1	3.6	10.2		8.9		
Aspergillilic acid	5.5						
Barbituric acid	4.0						
Benzoic acid	4.2						
Betaine	1.9				13.9		
Boric acid	9.2						
Brucine					3.1		
Butyric acid	4.8						

¹ The values recorded for such ampholytes as amino acids and their derivatives are calculated on the basis of the modern zwitter ion theory. These values (pk) are related to the classical values (pK) as follows: for monoamino acids, $pk_1 = 14 - pK_{OH}$, and $pk_2 = pK_H$; for diamino acids, $pk_1 = 14 - pK_{OH_2}$, $pk_2 = pK_{OH_1}$, and $pk_3 = pK_H$; for dicarboxylic amino acids, $pk_1 = 14 - pK_{OH}$, $pk_2 = pK_{H_1}$, and $pk_3 = pK_{H_2}$.

ACID-BASE RELATIONS

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TABLE 1 (Cont.)

	pK _{acid} (pK _H)				pK _{base} (pK _{OH})		
	1	2	3	4	1	2	3
Caffeine					13.4		
Canaline	2.4				3.7	9.2	
Canavanine	2.5				4.6	9.3	
Caproic acid	4.9						
Caprylic acid	4.9						
Carbonic acid	6.5 ¹	10.3					
Carnosine	2.6				6.8	9.5	
Catechol	9.1	12.1					
Cholic acid	4.9						
Cinchonine					6.8	9.5	
Cinnamic acid (<i>trans</i>)	4.4						
Cinnamic acid (<i>cis</i>)	4.9						
Citric acid	3.1	4.7	5.4				
Creatine	2.6				0		
Creatinine					9.2		
Crotonic acid (<i>trans</i>)	4.7						
Cysteine	2.0	10.3			8.2		
Cysteinylcysteine	2.65	9.35	10.85		7.3		
Cystine	1.2	1.7			7.5	9.0	
Cytidine					4.2		
Cytidylic acid	0.8	6.0			4.2		
Cytosine	12.2				4.6		
Dehydrocholic acid	4.9						
Desoxycholic acid	4.9						
Dihydroxyacetonephosphoric acid	1.8	6.45					
3,4-Dihydroxyphenylalanine	2.4	9.9	11.7		8.7		
Diiodotyrosine	2.1	6.5			7.8		
Dimethylamine					3.3		
Estriol	9.1						
Estrone	9.4						
Ethyl alcohol	13.8						
Formic acid	3.8						
Fructose	11.7						
Fructose-6-phosphoric acid	1.0	6.1					
Fumaric acid	3.0	4.5					
Galactose-1-phosphoric acid	1.0	6.2					
Gallic acid	4.3	8.9					
Glucose	12.1						
Glucose-1-phosphoric acid	1.1	6.1					
Glucose-6-phosphoric acid	0.95	6.1					
Glutamic acid	2.2	4.3			9.7		
Glutamine	2.2				9.1		

¹ This is the apparent constant based upon carbonic acid plus carbon dioxide. The true pK_{H1} for carbonic acid is 3.6.

TABLE 1 (Cont.)

	pK _{acid} (pK _H)				pK _{base} (pK _{OH})		
	1	2	3	4	1	2	3
Glutathione	2.1	3.5	9.1		8.7		
Glyceraldehydphosphoric acid	2.1	6.75					
Glycerol	14.2						
Glycerophosphoric acid	1.4	6.4					
Glycine	2.3				9.6		
Glycocholic acid	4.4						
Glycodesoxycholic acid	4.0						
Glycylaspartic acid	2.8	4.5			8.6		
Glycylglycine	3.1				8.1		
Glycylproline	2.8				8.5		
Glycyltyrosine	3.0	10.4			8.4		
Guanine	10.1				2.9		
Guanosine	9.2				1.6		
Guanylic acid	0.7	5.9	9.4		2.3		
Hemoglobin	8.2						
Hexosediphosphoric acid	1.5	6.3					
Hippuric acid	3.6						
Histamine					4.3	8.1	
Histidine	1.8				6.0	9.2	
Histidylglycine	2.4				5.8	7.8	
Histidylhistidine	2.25				5.6	6.8	7.8
Hydantoic acid	3.8						
Hydantoin	9.1						
Hydrocyanic acid	9.1						
Hydrogen peroxide	11.6						
Hydroquinone	9.9	12.0					
Hydrosulfuric acid	7.1	14.9					
<i>m</i> -Hydroxybenzoic acid	4.1	9.8					
<i>p</i> -Hydroxybenzoic acid	4.5	9.1					
<i>β</i> -Hydroxybutyric acid	4.8						
<i>β</i> -Hydroxyglutamic acid	2.3	4.2			9.6		
Hydroxylysine	2.1				8.6	9.7	
Hydroxyproline	1.9				9.7		
Hydroxyvaline	2.6				9.7		
Hypobromous acid	9.0						
Hypochlorous acid	6.0						
Hypoxanthine	8.7						
Imidazolelactic acid	3.0				6.7		
3-Indoleacetic acid	4.6						
3-Indolepropionic acid	4.8						
Inosine	8.7						
Inosinic acid	1.5	6.0	8.9				
Iodic acid	0.7						
Isoleucine	2.4				9.7		
Lactic acid	3.8						

ACID-BASE RELATIONS

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TABLE 1 (Cont.)

	pK _{acid} (pK _H)				pK _{base} (pK _{OH})		
	1	2	3	4	1	2	3
Lactose	12.2						
Leucine	2.4				9.6		
Levulinic acid	4.6						
Lithocholic acid	5.0						
Lysine	2.2				9.0	10.5	
Lysylglutamic acid	2.9	4.5			7.8	10.5	
Lysyllysine	2.0				8.2	9.5	10.6
Maleic acid	1.8	6.6					
Malic acid	3.4	5.0					
Maltose	11.7						
Mandelic acid	3.4						
Methionine	2.3				9.2		
Methylamine					3.7		
Methylmercaptan	10.7						
Methyl red					11.5		
Mucic acid	3.2						
Nicotinic acid	4.9						
Nitrous acid	3.4						
Norleucine	2.4				9.8		
Ornithine	1.9				8.7	10.8	
Oxalic acid	1.2	4.1					
Oxyhemoglobin	6.6						
Pantothenic acid	4.4						
Penicillin	2.3	3.5					
Phenaceturic acid	3.7						
Phenol	9.9						
Phenolphthalein	9.7						
Phenylacetic acid	4.3						
Phenylalanine	1.8				9.1		
Phenylalanylarginine	2.7				7.6	12.4	
Phenylalanylglycine	3.1				7.7		
Phenylhydrazine					8.8		
Phloroglucinol	8.4	8.9					
Phosphocreatine		2.7	4.6		2.0		
Phosphopyruvic acid		3.5	6.4				
Phosphoric acid	2.0	6.8	12.4				
Picric acid	0.8						
Proline	2.0				10.6		
Propionic acid	4.9						
Pyridine					8.8		
Pyridoxin					9.2		
Pyrogallol	10.0	11.6					
Pyrophosphoric acid	0.9	2.0	6.5	8.4			
Pyruvic acid	2.5						
Quinic acid	3.6						

TABLE 1 (Cont.)

	pK _{acid} (pK _H)				pK _{base} (pK _{OH})		
	1	2	3	4	1	2	3
Quinine					6.7	9.5	
Resorcinol	9.1	11.3					
Salicylic acid	3.0	12.4					
Sarcosine	2.2				10.0		
Serine	2.2				9.2		
Strychnine					7.0	10.2	
Succinic acid	4.2	5.6					
Sucrose	12.6						
Sulfadiazine	6.4						
Sulfanilamide	10.5				11.8		
Sulfapyridine	8.5						
Sulfathiazole	6.8						
Sulfuric acid		1.9					
Sulfurous acid	1.9	7.2					
Tannic acid	6.0						
Tartaric acid	3.0	4.5					
Taurine	1.5				8.7		
Taurocholic acid	1.6						
Taurodesoxycholic acid	1.9						
Theobromine					13.3		
Thiosulfuric acid	2.0						
Threonine	2.2				9.0		
Thymine	9.9						
Trichloroacetic acid	0.8						
Trimethylamine					4.3		
Tryptophane	2.4				9.4		
Tyramine					10.1		
Tyrosine	2.2	10.2			9.1		
Tyrosylarginine	2.6	9.8			7.5	12.3	
Tyrosyltyrosine	3.5	9.8	10.3		7.7		
Uracil	9.5						
Urea					13.8		
Uric acid	5.7	9.0					
Uridine	9.2						
Uridylic acid	1.0	5.9	9.4				
Valeric acid	4.8						
Valine	2.3				9.6		
Xanthine	9.9				13.2		
Xanthosine	6.0						

For those unfamiliar with logarithms, a brief explanation is given. All logarithms are exponents; the common logarithm of a number is the exponent which, applied to the base 10, gives that number. Thus, $100 = 10^2$, and 2 is defined as the common logarithm of 100.

$10^{0.00} =$	1,	Therefore the logarithm of	1 is 0
$10^{1.00} =$	10,	Therefore the logarithm of	10 is 1
$10^{2.00} =$	100,	Therefore the logarithm of	100 is 2
$10^{3.00} =$	1000,	Therefore the logarithm of	1000 is 3

(Any number raised to the zero power is one by the theory of exponents.) Every number between 1 and 10 has a corresponding log (abbreviation for logarithm) between 0 and 1. Between 10 and 100 every number has a corresponding log between 1 and 2, and so forth.

A logarithm consists of two parts, the characteristic to the left of the decimal point and the mantissa to the right. The mantissa depends only on the sequence of digits in the number. The characteristic depends entirely on the location of the decimal point in the number whose log is desired. If the number lies between 0 and 10, but not including 10, the characteristic is 0; between 10 and 100, but not including 100, the characteristic is 1. The rule is: take for the characteristic of the log of any number (one or greater) one less than the number of digits to the left of the decimal point.

$10^{0.0828} =$	1.21	The logarithm of	1.21 is 0.0828
$10^{1.0828} =$	12.1	The logarithm of	12.1 is 1.0828
$10^{2.0828} =$	121	The logarithm of	121 is 2.0828

The logs of 1.21, 12.1, and 121 all have the same mantissa; the decimal point determines the characteristic. Table 2 does not, therefore, give the characteristics but only the mantissas of the numbers. The characteristic for a number less than unity is negative and equal to one more than the number of zeros between the decimal point and the first non-zero digit.

$10^{-1.00} =$	$\frac{1}{10} = 0.1$	— 1.0 is the logarithm of 0.1
$10^{-2.00} =$	$\frac{1}{100} = 0.01$	— 2.0 is the logarithm of 0.01
$10^{-3.00} =$	$\frac{1}{1000} = 0.001$	— 3.0 is the logarithm of 0.001

Example: Find the logarithm of 0.121. From the foregoing it is seen that the characteristic is -1 . The table, however, only gives the *positive* mantissa, 0.0828. To obtain the complete logarithm, the characteristic -1 and the mantissa $+0.0828$ are added, algebraically, giving the negative logarithm -0.9172 in which both mantissa and characteristic are negative. For the number 0.0121 the logarithm -1.9172 is obtained in a similar manner.

When numbers are to be multiplied, their logs are added. When numbers are to be divided, the logarithm of the divisor is subtracted from the logarithm of the dividend. These relations are evident from the following examples.

$10^1 \times 10^1 = 10^2 =$	100	$10^1 \div 10^1 = 10^0 =$	1
$10^2 \times 10^1 = 10^3 =$	1000	$10^2 \div 10^1 = 10^1 =$	10
$10^2 \times 10^3 = 10^5 =$	100,000	$10^3 \div 10^2 = 10^1 =$	10

Multiply 1.36 by 11.3

Log 1.36	= 0.1335
Log 11.3	= <u>1.0531</u>
Log of product	= 1.1866
Product	= 15.37

Divide 11.3 by 1.36

Log 11.3	= 1.0531
Log 1.36	= <u>0.1335</u>
Log of quotient	= 0.9196
Quotient	= 8.31

TABLE 2
LOGARITHMS

Natural Num- bers	0	1	2	3	4	5	6	7	8	9	PROPORTIONAL PARTS								
											1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3	6	8	11	14	17	20	22	25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	6	7	7	8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7

TABLE 2 (Cont.)

LOGARITHMS

Natural Num- bers	0	1	2	3	4	5	6	7	8	9	PROPORTIONAL PARTS								
											1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	4	4

In place of writing either very large or very small numbers, it is desirable, for brevity and quick comprehension, to express them as integers multiplied by ten with the correct exponent. Given the number 0.0000136. Since $0.00001 = 10^{-5}$, $0.0000136 = 1.36 \times 10^{-5}$. Similarly, $0.000000263 = 2.63 \times 10^{-7}$. Note, also, that $1.36 \times 10^{-5} = 0.136 \times 10^{-4}$ or 13.6×10^{-6} . It is usual to express such numbers with one integer to the left of the decimal point. Thus, $21,000,000 = 2.1 \times 10^7$.

To determine the logarithm of the square of a number greater than unity, multiply the logarithm of the number by 2; to determine the square root of a number greater than unity, divide the logarithm of the number by 2.

$$\begin{array}{ll} \text{Examples: } \log 24^2 = 2 \times \log 24 & \log \sqrt{289} = \frac{1}{2} \log 289 \\ & = \frac{1}{2} (2.4609) \\ & = 2.7604 & = 1.2304 \end{array}$$

$$\text{Hence, } 24^2 = 576 \qquad \text{Hence, } \sqrt{289} = 17$$

To find the square of 8.1×10^{-7} :

$$\begin{array}{l} 2 \times \log 8.1 = 2 \times 0.9085 = 1.8170; \text{ hence, } 8.1^2 = 65.61 \\ 2 \times \log 10^{-7} = 2 \times -7 = -14; \text{ hence, } (10^{-7})^2 = 10^{-14} \end{array}$$

$$\text{Hence, } (8.1 \times 10^{-7})^2 = 65.6 \times 10^{-14} = 6.56 \times 10^{-13}$$

To find the square root of 8.1×10^{-7} , convert the number to 81×10^{-8} , so that the exponent of 10 is exactly divisible by 2:

$$\begin{array}{l} \log \sqrt{81} = \frac{\log 81}{2} = \frac{1.9085}{2} = 0.9542; \text{ hence, } \sqrt{81} = 9 \\ \log \sqrt{10^{-8}} = \frac{-8}{2} = -4; \text{ hence, } \sqrt{10^{-8}} = 10^{-4} \\ \sqrt{8.1 \times 10^{-7}} = 9 \times 10^{-4} \end{array}$$

IONIZATION OF WATER

Water ionizes slightly into hydrogen and hydroxyl ions, and at equilibrium,

$$\frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} = K$$

The actual quantity of hydrogen ions in water at 24°C ., as determined by conductivity methods, is 0.0000001 N or $1 \times 10^{-7} \text{ N}$. The concentration of hydroxyl ions is the same, for $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$. Since only one part of water in each ten million parts is ionized, we may consider the $[\text{H}_2\text{O}]$ in the above equilibrium equation as constant; the equation may then be written $[\text{H}^+] \times [\text{OH}^-] = K_w$. Hence, K_w equals $(1 \times 10^{-7}) \times (1 \times 10^{-7})$, or 1×10^{-14} , and its negative logarithm, or $\text{p}K_w$, is 14. The constancy of this ionization product for water implies that in aqueous acid solutions where the $[\text{H}^+]$ has been increased by dissociation of the hydrogen of the added acid, there will be a proportionate decrease in

[OH⁻]; but the ionization product will always be 1×10^{-14} , at 24° C. At body temperature (37° C.) it is $1 \times 10^{-13.60}$. Water is neutral because the concentrations of ionized hydrogen and hydroxyl are equal.

The term pH is defined as

$$\log \frac{1}{[\text{H}^+]}$$

or $\log 1 - \log [\text{H}^+]$. Since $\log 1 = 0$, the $\text{pH} = 0 - \log [\text{H}^+]$ or $-\log [\text{H}^+]$, i.e., the negative log of the hydrogen ion concentration.

To express a $[\text{H}^+]$ of 1.36×10^{-4} as pH, it is necessary to find the negative log of 1.36×10^{-4} . The log of 10^{-4} is -4 , the log of 1.36 (from the tables) is $+0.1335$, and since this is a product, the logs are added. The log of 1.36×10^{-4} is therefore -3.8665 , and the pH is $-(-3.8665)$ or 3.8665.

To express a pH of 4.6271 as $[\text{H}^+]$: $4.6271 = -\log [\text{H}^+]$. Hence, $(-4) + (-0.6271) = (-5) + (+0.3729)$. (To use Table 2, a positive mantissa is necessary.) From the table, 0.3729 is found to be the log of 2.36, while -5 is the log of 10^{-5} . Hence, the $[\text{H}^+]$ is 2.36×10^{-5} .

In Table 3, the approximate dissociations of several acids are given. 0.001 N acetic acid is 13.6 per cent dissociated. To find the ionization constant: the $[\text{H}^+]$ is $(1 \times 10^{-3}) \times (1.36 \times 10^{-1})$, or 1.36×10^{-4} ; the $[\text{CH}_3\text{COO}^-]$ is obviously equal to the $[\text{H}^+]$; and, according to the mass law,

$$K = \frac{[\text{H}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} = \frac{(1.36 \times 10^{-4})(1.36 \times 10^{-4})}{1 \times 10^{-3}} = \frac{1.85 \times 10^{-8}}{1 \times 10^{-3}} = 1.85 \times 10^{-5}$$

To find the pK:

$$\log 10^{-5} = -5.00$$

$$\log 1.85 = 0.27$$

$$(-5) + (0.27) = -4.73$$

Hence,

$$\text{pK} = 4.73$$

TABLE 3

PER CENT DISSOCIATION

	1.0 N	0.1 N	0.01 N	0.001 N
Acetic acid	0.4	1.4	4.3	13.6
Ammonium hydroxide	0.6	1.9	5.9	19.0
Citric acid	(4.5) ¹	(14.9)	(38.5)	
Hydrochloric acid	78.0	91.0	96.0	98.0
Lactic acid	(1.1)	(3.7)	(11.0)	(30.9)
Potassium hydroxide	(77.0)	(95.0)	(100.0)	(100.0)
Sodium hydroxide	73.0	84.0	92.0	96.0
Sulfuric acid	(51.0)	(65.0)	(86.0)	(99.0)

¹ Values in parentheses are calculated.

The pH of water at 24° C. is 7.0; an acid solution has a pH lower than 7.0. Decreasing pH means increasing $[H^+]$. Since the pH scale is logarithmic, a pH change of 1.0 represents a tenfold change in hydrogen ion concentration. The relations of acidity and alkalinity to pH and pOH are illustrated further in Table 4.

TABLE 4
pH AND pOH OF SOLUTIONS

pH	Solution	pOH
0.0	HCl, 1.0 N	14.0
1.0	HCl, 0.1 N	13.0
2.0	HCl, 0.01 N	12.0
2.9	CH ₃ COOH, 0.1 N	11.1
3.0	HCl, 0.001 N	11.0
4.7	NaH ₂ PO ₄ ¹	9.3
4.7	$\frac{CH_3COONa}{CH_3COOH} = 1$	9.3
7.0	H ₂ O	7.0
7.2	$\frac{Na_2HPO_4}{NaH_2PO_4} = 1$	6.8
7.4	$\frac{NaHCO_3}{H_2CO_3} = \frac{20}{1}$	6.6
8.4	NaHCO ₃ ¹	5.6
9.7	Na ₂ HPO ₄ ¹	4.3
11.0	NaOH, 0.001 N	3.0
11.1	NH ₄ OH, 0.1 N	2.9
12.0	NaOH, 0.01 N	2.0
12.3	$\frac{Na_2PO_4}{Na_2HPO_4} = 1$	1.7
13.0	NaOH, 0.1 N	1.0
14.0	NaOH, 1.0 N	0.0

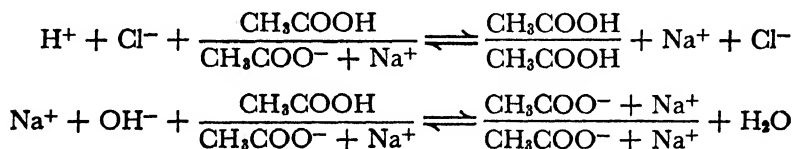
¹ The pH of a sodium bicarbonate solution can be calculated from the equation: $pH = \frac{1}{2}(pK_1 + pK_2) = \frac{1}{2}(6.5 + 10.3) = 8.4$. This relation applies within rather wide limits of normality. In a similar way, a solution of monosodium phosphate has a pH of $\frac{1}{2}(2.16 + 7.16)$ or 4.7, and the pH of disodium phosphate solution is $\frac{1}{2}(7.16 + 12.3)$ or 9.7.

The pH of a solution may be determined either by the use of indicators or by an electrometric method. The indicator method is extensively employed for the approximate determinations frequently desired in biological work; it will be described following a consideration of buffer solutions. The electrometric method is a more accurate procedure employed

by physical chemists and research workers. Articles describing this method are available in the references. Although hydrogen, quinhydrone, antimony, and glass electrodes have all been employed, the latter is most often used in biological laboratories.

BUFFER SOLUTIONS

A buffer solution is one which tends to maintain its hydrogen ion concentration when appreciable amounts of acid or base are added. Thus, a small drop of N hydrochloric acid added to a liter of pure water will change the hydrogen ion concentration approximately two hundred times, but the same quantity of acid or alkali produces very little change in an efficient buffer solution. Buffer solutions contain a slightly dissociated acid and its salt, or a slightly dissociated base and its salt. The behavior of buffer systems may be illustrated by the following equations:



The more highly dissociated acid or alkali (written in the ionized state) reacts with the buffer system. Actual removal of hydrogen ions in the one case, and of hydroxyl ions in the other, occurs. There is a tendency to maintain the original pH of the buffer solution.

The buffer mechanism allows neutralization of comparatively large amounts of acid or alkali with little change in the pH of the solution; yet there are limits to the effectiveness of buffer solutions, for it is obvious that one component of the original buffer system is converted into the other during neutralization, and the ratio

$$\frac{\text{HA}}{\text{A}^-}$$

is changed by the neutralizing reaction. This ratio is related to the hydrogen ion concentration of the buffer solution as follows:

$$\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = K, \quad \text{or} \quad [\text{H}^+] = K \frac{[\text{HA}]}{[\text{A}^-]}$$

Since HA is largely undissociated while its salt, BA, is highly ionized, the $[\text{A}^-]$ of the equation is nearly identical with $[\text{BA}]$. Substituting BA for A^- , we have:

$$[\text{H}^+] = K \frac{[\text{HA}]}{[\text{BA}]}$$

This may be expressed in terms of pH and pK as follows:

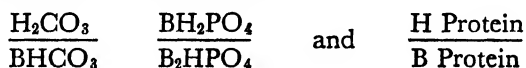
$$\frac{1}{[H^+]} = \frac{1}{K} \times \frac{[BA]}{[HA]}$$

$$pH = pK + \log \frac{[BA]}{[HA]} \text{ (Henderson-Hasselbalch equation)}$$

Inasmuch as the hydrogen ion concentration of the buffer solution is primarily dependent upon the ratio of the components of the buffer system, different ratios can be used to prepare standard buffer solutions of definite pH. In buffer solutions which contain equimolecular proportions of the two components,

$$pH = pK \times \log \frac{[1]}{[1]}, \text{ or } pH = pK$$

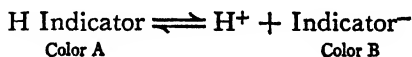
This relation is useful for calculating the pH of a buffer mixture from the ionization constant of the weak acid component. Buffer solutions may be diluted somewhat without causing much change in either the ratio of the constituents or in the pH. Some of the more important buffer systems in tissues and body fluids are the following:



In the laboratory there is frequent need for buffers in biological and enzymatic experiments, and as standards for the colorimetric determination of pH. The buffers for colorimetric standards are usually acetate, borate, glycine, phosphate, or phthalate mixtures.

INDICATORS

The usual laboratory indicators are slightly ionizable organic acids or bases which exhibit different colorations when ionized and un-ionized, thus:



The ionization equation for an indicator acid would be:

$$\frac{[H^+][\text{Indicator}^-]}{[H \text{ Indicator}]} = K \quad \text{or} \quad pH = pK + \log \frac{[\text{Indicator}^-]}{[H \text{ Indicator}]}$$

Since the ionization constant is fixed for any given indicator, it is evident that the ratio between the colored forms of the indicator will depend upon and be a measure of the hydrogen ion concentration, or pH. For example, undissociated phenol red is yellow at pH 6.8, and its ionized form, at pH 8.4, is red; at pH 7.4 it will have an orange shade. By adding phenol

red to a graded series of standard buffer solutions of pH 6.8 to 8.4, a series of standards is available whose colors differ visibly and progressively. The color of the same concentration of phenol red in an unknown solution can be compared quickly with this series to determine the pH of the unknown. It is, of course, necessary to choose pH indicators having effective color variations over the pH range to be investigated (Table 5). While the colorimetric method affords a rapid and convenient determination of hydrogen ion concentration, it is not as accurate as electrometric methods.

TABLE 5
INDICATORS AND BUFFER STANDARDS

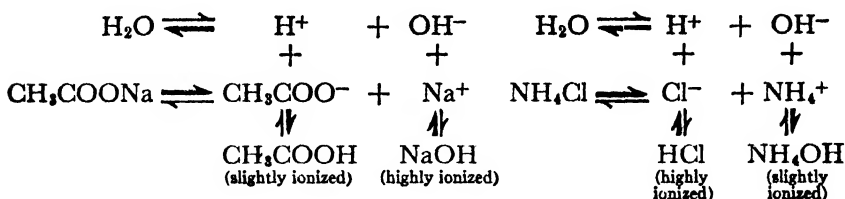
INDICATORS		pH RANGE
<i>pH</i>		
Metacresol purple	(Red)	1.2 to 2.8 (Yellow)
Thymol blue	(Red)	1.2 to 2.8 (Yellow)
Bromphenol blue	(Yellow)	3.0 to 4.6 (Blue)
Methyl red	(Red)	4.2 to 6.3 (Yellow)
Bromcresol purple	(Yellow)	5.2 to 6.8 (Purple)
Bromthymol blue	(Yellow)	6.0 to 7.6 (Blue)
Phenol red	(Yellow)	6.8 to 8.4 (Red)
Neutral red	(Red)	6.8 to 8.0 (Yellow)
Thymol blue	(Yellow)	8.0 to 9.6 (Blue)
<i>o</i> -Cresolphthalein	(Colorless)	8.3 to 9.8 (Red)
Alizarin yellow R	(Yellow)	10.0 to 12.0 (Red)
2,4,6-Trinitrobenzene	(Colorless)	12.0 to 14.0 (Red)
<i>Titration</i>		
Töpfer's reagent	(Red)	2.9 to 4.0 (Yellow)
Methyl orange	(Red)	3.1 to 4.4 (Yellow)
Alizarin red ¹	(Yellow)	5.0 to 6.8 (Red)
Phenolphthalein	(Colorless)	8.2 to 10.0 (Red)
Thymolphthalein	(Colorless)	9.3 to 10.5 (Blue)
<i>Qualitative</i>		
Congo red	(Blue)	3.0 to 5.0 (Red)
Litmus	(Red)	4.5 to 8.5 (Blue)
Nitrazine ²	(Yellow)	4.5 to 7.5 (Blue)
BUFFER STANDARDS		
KCl + HCl		1.2 to 2.2
KH-Phthalate + HCl		2.2 to 3.8
KH-Phthalate + NaOH		4.0 to 6.2
KH ₂ PO ₄ + NaOH		5.8 to 8.0
KCl + NaOH + H ₃ BO ₃		7.8 to 10.0

¹ Sodium alizarin sulfonate.

² This test paper, which gives a graded series of colors over the pH range indicated, is useful for determining urinary pH.

TITRATABLE ACIDITY

Almost all salts are considered to be highly ionized in aqueous solution. Pure water, as has been noted, ionizes to a slight extent. The reaction of a salt solution may be neutral, acid, or basic, depending upon the nature of the salt and its hydrolytic relations (salt hydrolysis) illustrated by the equilibria:



In a sodium acetate solution some of the acetate ions combine with hydrogen ions from the water to form acetic acid, leaving a preponderance of hydroxyl ions in the solution; the pH of the solution is, therefore, greater than 7. In ammonium chloride solutions this result is reversed. The hydrogen and hydroxyl ions approximately balance in sodium chloride solutions. These relations are visualized in the titration curves of Figure 1, which show the changes in pH during neutralization. Such curves are accurately plotted from electrometric data, or they can be determined by calculations based on the mass law. They are of considerable assistance in determining the indicator which will give the best results.

If 100 ml. of 0.1 N NaOH are added to 100 ml. of 0.1 N acetic acid the pH of the solution will be 8.87. This is the equivalence or end point on the titration curve, and the indicator used must show a decided color change in that pH range. For example, methyl orange is not applicable as only 22 per cent of the acid is neutralized at pH 4.2, and there is a titration error of 78 per cent. Similarly, methyl red begins to change color at pH 4.6, where not even 50 per cent of the acid is neutralized; and it is yellow at pH 6 when about 93 per cent is neutralized. The titration error is minimal when phenolphthalein is used. This indicator will show a sharp color change at the equivalence point.

When the titration curve becomes horizontal or flattens out in the vicinity of the equivalence point, it indicates that a very small addition of base produces a decided increase in pH, and makes possible a sharp end point with an indicator which changes color in that pH range. The titration curves of carbonic and phosphoric acids show that excellent buffer systems are formed and that sharp end points are unobtainable. In general, titration curves show that weak acids should be titrated with phenolphthalein, thymolphthalein, or thymol blue; and weak bases with methyl red or methyl orange.

As the pK of the acid increases, the changes in pH at equivalence become less, and hydrolysis of the anion is more pronounced. If the pK is greater than 7, the acid cannot be satisfactorily titrated in 0.1 N solu-

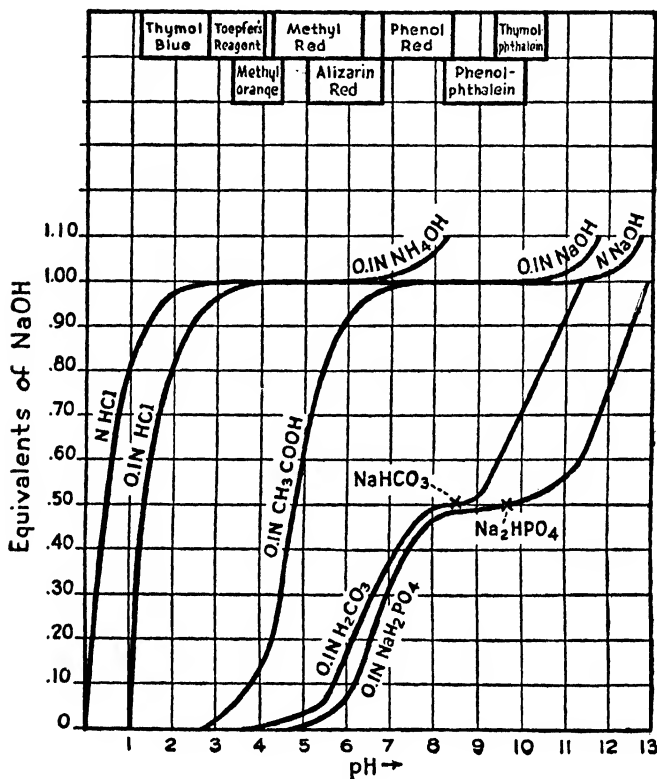


FIG. 1. Titration curves.

tion. When an acid contains more than one replaceable hydrogen atom it can be titrated both as a univalent and divalent acid, provided the ratio

$$\frac{K_1}{K_2}$$

is greater than 10^4 . This ratio is less than 10^4 for carbonic acid which can be determined only as a monobasic acid by direct titration. The same general considerations apply to bases.

METABOLISM

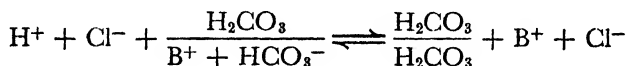
"Sciences begin with what might be called the natural history stage (mere description), but as knowledge increases, analytical and experimental methods are introduced and the relations between phenomena are formulated into ever wider laws." — MORRIS R. COHEN

BUFFERS OF BLOOD AND LYMPH

Bicarbonate, phosphate, and protein buffer systems are widely distributed in tissues and body fluids. The pH of blood is maintained near 7.4 by buffers, which confer on this fluid 20,000 times the neutralizing capacity of a sodium hydroxide solution of identical pH. The



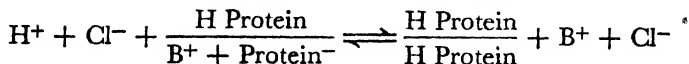
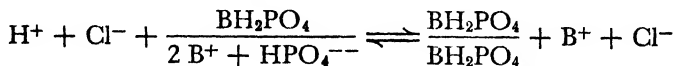
system is the simplest buffer of blood plasma (the fluid portion of the blood as distinguished from the cellular elements). Glands of the stomach secrete approximately 0.1 N hydrochloric acid, some of which is subsequently neutralized in the small intestine by secretions which may be regarded as filtered blood plasma. The bicarbonate buffer of blood plasma reacts as follows:



Note the familiar transformation of hydrogen ions into feebly ionized carbonic acid, with adjustment of the pH of the acid gastric juice toward 7.4 (the pH of normal plasma) and a corresponding change in the ratio,



The phosphate and protein buffer systems of plasma also react with hydrochloric acid, as follows:



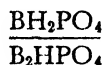
The concentration of bicarbonate buffer is approximately equal to 60 ± 10 volumes per cent of total carbon dioxide in normal venous blood, and 52 volumes per cent in arterial blood. The physiological superiority of this buffer is due to the fact that carbonic acid is readily converted to water and volatile carbon dioxide in the pulmonary circulation. The velocity of this reversible reaction is accelerated by a zinc-containing

enzyme, *carbonic anhydrase*, present in the erythrocytes, gastric mucosa (chiefly in the parietal cells), renal cortex, liver, pancreas, muscle, cerebrum, lens, and retina. The enzyme appears in the eyecup early in development, and in the blood of the chick at the mid-point of embryonic life. The quantity of carbonic anhydrase in adult mammalian blood is approximately 100 mg. per cent; it parallels the erythrocyte count, except in jaundice, when it is independently increased. In infant blood the carbonic anhydrase concentration is only 50 mg. per cent, and it is even lower in premature infants and at times in infant cyanosis. Thiocyanate, sulfide, cyanide, and sulfanilamide inhibit the activity of carbonic anhydrase. Inhibition of gastric secretion of hydrochloric acid in dogs by sulfanilamide indicates that carbonic anhydrase activity is essential for this process. The enzyme is also involved in egg shell formation in hens.

The maximal effect of the bicarbonate system is at pH 6.1, where the ratio of its components is 1; while the phosphate buffer system exhibits its maximal effect at pH 6.8. Normal plasma has a pH of 7.4, with a



ratio of $\frac{1}{20}$, and a



ratio of approximately $\frac{1}{4}$. The phosphate buffer of plasma is present in rather low concentration (4 mg. of phosphorus per 100 ml.); greater concentrations exist in the cells and tissues. In plasma, the protein buffers are quantitatively more important than phosphates, since the protein concentration is approximately 7 gm. per 100 ml.

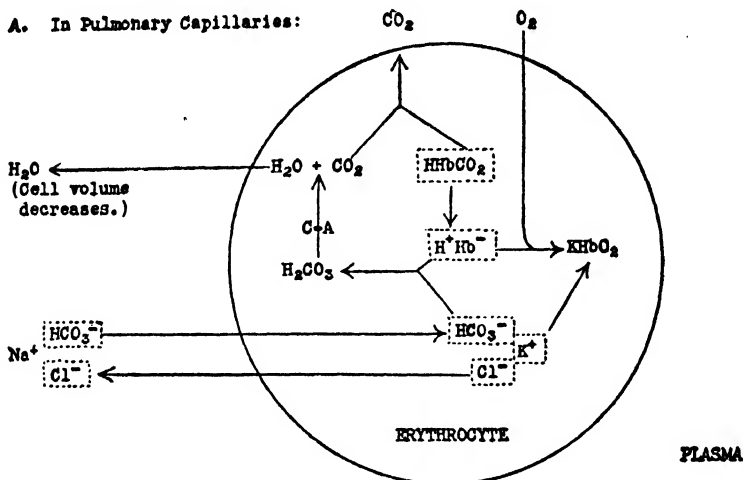
HEMOGLOBIN AS SPECIAL NEUTRALITY REGULATOR

Hemoglobin and its oxygenated form, oxyhemoglobin, compose 34 per cent of the erythrocytes of the adult man, and they play a very important triple role in the buffering mechanism of the blood. They assist neutralization by ordinary buffer action, by liberation of base when oxyhemoglobin is reduced, and by direct combination with carbon dioxide to form carbohemoglobin. Hemoglobin is an amphoteric protein; it acts as a buffer over a more extended range than either bicarbonate or phosphate, because it contains acid radicals which overlap in their effects.

Hemoglobin is converted with ease to oxyhemoglobin in the pulmonary capillaries, and it normally transports oxygen from the lungs to other tissue capillaries where it is again partially changed to hemoglobin. This reaction is accelerated by carbon dioxide entering from the tissues. (Consult Figure 2, where hemoglobin is indicated as HHb and oxyhemoglobin as HHbO₂.) Oxyhemoglobin (pK = 6.62) is a more highly dissociated acid

than hemoglobin ($pK = 8.18$). At the pH of erythrocytes, oxyhemoglobin combines with approximately 55 per cent more base than does hemoglobin.

A. In Pulmonary Capillaries:



B. In Tissue Capillaries:

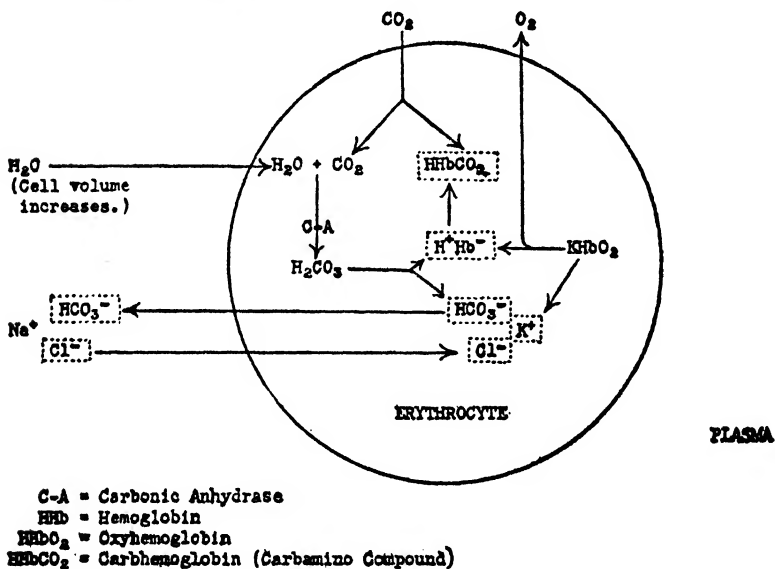


FIG. 2. Chloride or anion migration.

The decreased base-combining power of the hemoglobin, formed in the capillaries, greatly assists in the neutralization of normal acid metabolic

products diffusing into the capillaries from the surrounding tissue spaces. The pH of normal venous blood is only slightly lower than that of arterial blood; the plasma changes by 0.03 pH, the cells by 0.02 pH. Conversely, the influx of acid metabolites (end products of cellular metabolism) compensates for the relative alkalinity of the hemoglobin formed in the capillaries. The erythrocyte could conceivably maintain its pH during circulation if the cations which are combined with the non-diffusible oxyhemoglobin¹ could freely migrate outward into the surrounding plasma. Experiments with isotopes show that mammalian erythrocytic membranes are normally about fifty thousand times more permeable to anions than to most cations. One may, therefore, regard the erythrocytic membrane as relatively impermeable to cations.

The student should trace the balancing phenomena occurring in lung capillaries, and their reversal in tissue capillaries as outlined in Figure 2. This series of exchanges between erythrocytes and plasma is called the *anion migration* or *chloride shift*. The movement of bicarbonate is accompanied by a reverse movement of chloride anion and osmotically attracted water. As a result, the cell volume of erythrocytes increases in the tissue capillaries and decreases in the lung capillaries. Stasis of blood is undesirable when samples are collected for determination of blood or corpuscular volume, since this may lead to an abnormal carbon dioxide content and increased volume of the erythrocytes.

DONNAN EQUILIBRIUM

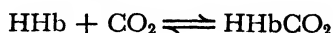
Ionic exchanges through the walls of cells are explained by physical chemists in terms of the Donnan theory of membrane equilibrium. The theory deals with membranes that are impermeable to certain ions, as, for example, protein ions. Under these circumstances the products of the diffusible ions at each side of the membrane will be equal when equilibrium is attained; but their individual concentrations will be unequal owing to unequal distribution of protein ions. This effect is partly responsible for differences in pH, electrical potential and osmotic pressure between the interior and exterior of cells. Donnan equilibria also exist between different intracellular areas. It is not necessary to have an extraneous membrane; differing adjacent colloidal gels suffice.

CARBON DIOXIDE TRANSPORT

The chloride shift, which accompanies the reversible oxygenation of hemoglobin, allows the transportation of twice as much acid from the tissues to the lungs as does the pure buffering capacity of hemoglobin, and more than ten times as much as the plasma protein buffers. The total carbon dioxide content of venous blood is normally from 5 to 10 volumes per

¹ Proteins do not diffuse easily through membranes, as explained in Chapter II.

cent more than that of arterial blood. The anion migration is associated with at least 55 per cent of this transport, or one-half mol of carbon dioxide for each mol of oxygen exchanged via hemoglobin. Another 20 per cent of the carbon dioxide carriage seems to be due to the combination of carbon dioxide with hemoglobin to form a carbamino compound:



The carbon dioxide actually combines with an amino radical of the hemoglobin, as follows:



These relations are indicated in Figure 2. The carbamino reaction is rapid, entails no formation of H_2CO_3 and does not require carbonic anhydrase action. About 20 per cent of the carbon dioxide transport is traceable to simple buffer action of hemoglobin. In contrast to the importance of hemoglobin, the plasma buffers account for only 5 per cent of the total carbon dioxide transport, and phosphates are responsible for less than one tenth of this fraction. It is evident that marked reduction of plasma protein will have little effect on the buffering mechanisms of the blood.

The figures just quoted represent the carbon dioxide exchange. They should not be confused with the distribution of the total carbon dioxide in venous blood, which is as follows: 5 per cent as carbon dioxide in solution; 5 per cent as carbamino compound; and 90 per cent as bicarbonate.

pH OF BLOOD

The above mechanisms normally maintain the pH of plasma at an astonishingly constant value, namely, 7.4 ± 0.05 . The extreme variation during life is only $\pm \text{pH } 0.4$. Human blood is always slightly alkaline, for at the temperature of our bodies the pH of water is 6.8 (page 13). The pH of erythrocytes is approximately 7.3.

The neutralization of acid accomplished by the blood may be illustrated by the following examples: The addition of only enough hydrochloric acid to make a 0.000,001 M solution lowers the pH of water from 7.0 to 6.0, a result which would be fatal in the body. Also, the total carbon dioxide of normal blood, if dissolved in water alone, would produce a pH of 4.5. An adult man weighing 70 kilograms has 5 liters of circulating blood (7.6 per cent of the body weight), which contains 250 ml. of N carbon dioxide. It has previously been stated that approximately $\frac{1}{3}$ of this carbon dioxide is present as carbonic acid, the remainder being largely bicarbonate. To lower the pH of 5 liters of blood from 7.4 to 7.0 requires 150 ml. of N hydrochloric acid or 2400 ml. of carbon dioxide gas, a very appreciable neutralizing capacity. Also, beyond the circulating blood and in equilibrium with it, the tissues have five times as much alkali

available for rapid neutralization of acids as does the blood itself. The entire body can, therefore, rapidly dispose of approximately 6 times 150 or 900 ml. of normal involatile acid, the equivalent of 15 ml. per kg. of body weight. The cadaver of a diabetes mellitus patient, whose death was due to acid accumulation, has been shown to contain acetone acids equivalent to 100 grams or approximately 1 liter of N β -hydroxybutyric acid. With exhaustion of the neutralizing mechanisms of the body, the pH of the blood is lowered to a fatal level.

GENERAL IMPLICATIONS OF BUFFER ACTION IN THE BODY

The bicarbonate buffer system is present in tissue cells, but there it is quantitatively less important than in circulating fluids, such as blood plasma, lymph, tissue fluids, and secretions. Tissues contain more organic and inorganic phosphate and more protein than do body fluids. The cell protein buffer systems are effective over a considerable pH range. Table 6 shows that living cells are generally more acid than blood plasma. The pH of metabolically sluggish tissues is near 7.4, because these tissues form little acid by the oxidation of foods. When body fluids are infected

TABLE 6
APPROXIMATE pH OF TISSUES AND BODY FLUIDS

Bile (hepatic)	8.0 \pm 0.5
Colonic secretion	8.0
Pancreatic juice	7.8 \pm 0.3
Cell nuclei :	7.7 \pm 0.1
Intestinal juice (succus entericus)	7.4 \pm 0.3
Blood, lens, cerebrospinal, intra-ocular, peritoneal and synovial fluids	7.4
Mucus, tears :	7.3 \pm 0.3
Connective tissue, semen, tendons, transudates	7.2
Feces (adult) :	7.1 \pm 0.2
Serous exudates	7.0
Cell cytoplasm, nasal mucosa	7.0 \pm 0.2
Muscle juice, human milk	6.8 \pm 0.2
Saliva : : : :	6.7 \pm 0.4
Cow's milk	6.7
Pus serum :	6.6 \pm 0.6
Duodenal contents	6.5 \pm 0.5
Prostatic fluid	6.4 \pm 1.0
Urine . . : :	6.2 \pm 1.7
Perspiration : :	6.0 \pm 0.7
Feces (infant) :	6.0 \pm 1.0
Skin surface	5.5 \pm 1.5
Gastric juice (infant)	5.0
Vaginal mucosa	4.5 \pm 0.5
Gastric juice (adult)	1.2 \pm 0.3

with living micro-organisms they become more acid as the result of bacterial metabolic activity.

The low pH of urine indicates that the kidney is an important acid-excreting organ. Phosphates are normally the chief acid excretory products and principal buffers of the urine. The very low pH of normal gastric juice has a special secretory significance (page 135).

Interesting micromanipulation experiments have demonstrated that the protoplasm of cells is maintained near pH 6.9 by buffer systems. However, when one speaks of the pH of a cell an over-all average is implied. Actually, there are intracellular areas or particle surfaces with different reactions; the nucleus is relatively alkaline (approximately pH 7.7). Injection of acid into living cells stained with vital indicators has shown that maintenance of the pH is as necessary for cells as for blood plasma. Life and the normal function of cells are maintained by enzymes which are highly sensitive to pH changes. Knowledge of these relations is put to practical use in bacteriological laboratories in the control of the pH of bacterial media. Grossly demonstrable physiological functions of nerves, blood vessels, and muscles are also affected by changes in hydrogen ion concentration.

It is important to realize that the buffer and neutrality mechanisms so far considered achieve only temporary neutralization. Their activity entails displacement of buffer components which must be replaced to preserve the buffering efficiency of the body. Permanent disposal of excess acid or base is achieved through the excretory organs: lungs, kidneys, intestine, and sweat glands.

ORIGIN AND TRANSPORT OF ACIDS

Metabolic processes form acids by the oxidation of organic foodstuffs. Carbon dioxide is produced in large quantities from all organic foods, and the human adult must daily transport about 440 to 880 gm. of carbon dioxide, which is equivalent to 20 to 40 liters of N carbonic acid. The tissue cells are generally permeable to dissolved molecular carbon dioxide, but not to bicarbonate anion. Carbon dioxide is produced so rapidly and constantly by living tissues that any alkali which gains access to the body is quickly converted to bicarbonate. The circulating blood transports the bicarbonate rapidly from the general tissues to the alveoli of the lungs, whose gaseous reservoir contains approximately 5.6 per cent of carbon dioxide. The alveolar carbon dioxide is continually washed away by inspired air which contains only 0.025 per cent of this gas. The carbon dioxide tension of blood is intimately related to that of the alveolar air. The partial pressure of this gas in the arterial blood and alveolar air is normally 40 mm. of mercury, and in venous blood it is 46 mm. of mercury. Equilibrium is established during the one second in which the blood traverses the lung capillaries. The pH and carbon dioxide tension

of arterial blood control the respiratory rate. In order to increase ventilation in patients who have stopped breathing or who are poisoned with carbon monoxide, 5 per cent of carbon dioxide gas is mixed with the oxygen used for resuscitation. The respiratory center is remarkably sensitive to changes in plasma pH; a decrease of 0.012 in the pH of arterial blood doubles the alveolar ventilation. The center thus compensates for the entrance of acids into the blood stream by increasing the respiratory rate and volume. This relationship gives rise to an important clinical symptom of acidosis, namely, hyperpnea. The ratio of pulmonary ventilation to the carbon dioxide production in distant tissues is a prime factor in determining the pH of the blood. Since the carbon dioxide of blood exists largely as bicarbonate, fluctuations of the latter influence the pH of the respiratory center and, therefore, the rate of respiration.

In the normal cell, fats and carbohydrates are oxidized almost exclusively to water and carbon dioxide; oxidation of proteins produces carbonic acid and, in addition, appreciable amounts of involatile sulfuric and phosphoric acids, which arise from the metabolism of sulfur and phosphate radicals of the proteins. The sulfur of proteins exists chiefly in —S—C— linkage, but the phosphorus is present as preformed phosphate radicals esterified with the hydroxy amino acid, serine. Oxidation of 100 gm. of protein requires the excretion of 60 ml. of N sulfate and causes the withdrawal of 50 ml. of N base from the buffer systems.¹ A variety of other organic foods, such as nucleic acids, phospholipides, and sugar phosphate esters, also contain esterified phosphoric acid. Phosphate radicals that are set free from organic compounds may be considered equivalent to the ingestion of a similar quantity of acid phosphate (BH_2PO_4).

Organic acids, such as lactic, acetoacetic, and β -hydroxybutyric acids, are liberated by metabolic processes in living cells; the greater portion of these acids normally undergoes oxidation to carbonic acid and water. Accumulation of organic acids indicates partial oxidation or incomplete metabolism of the chief organic foods. For example, in diabetes mellitus organic acids are actively synthesized by chemical mechanisms which are out of physiological control. Blood and tissues contain small quantities of fatty acids, sugar acids, amino acids, nucleic acids, and so on, whose metabolism is reviewed in succeeding chapters; but the three organic acids mentioned above are the only ones of immediate interest in acid-base metabolism.

LACTIC ACID

Blood normally contains 15 ± 5 mg. per cent of lactic acid. Strenuous muscular activity can cause a temporary increase to as much as 130 mg.

¹ Customary values for total base or total cations in biological material do not include hydrogen ions.

As far as involatile cations are concerned, the organism can adjust its acid-base balance only by drawing on stores of these substances or by diminishing their excretion. Calcium is stored chiefly in the bones; sodium in body fluids, muscles, and bones; and potassium in muscles. As excess anions appear in the body, they combine with the base of body fluids and are excreted. Protoplasm is then destroyed, the cell volume decreases, and cations and phosphate are liberated from the cells to replace the loss from body fluids. Additional calcium and phosphate are also mobilized from bone. Not more than 3 per cent of the total base in the body can be used for rapid neutralization of acid; but much more can be utilized if the acid is administered or generated very slowly. Much of the calcium of the

TABLE 7
EFFECT OF FOODS UPON THE ACID-BASE BALANCE
OF THE BODY¹

ACID-ASH FOODS	ALKALINE-ASH FOODS
(Approximate Order of Acidifying Action)	(Approximate Order of Alkalinizing Action)
Crab (most acid)	Molasses (most basic)
Egg yolk	Olives
Lobster	Raisins
Scallops	Spinach
Liver	Beans
Beef	Almonds
Chicken	Avocados
Pork	Beets
Fish	Tomatoes
Oysters	Celery
Turkey	Carrots
Mutton	Cantaloupe
Wheat	Citrus fruits
Peanuts	Lettuce
Walnuts	Potatoes
Crackers	Turnips
Egg white	Bananas
Rice	Coconut
Bread	Cabbage
Cheese	Berries
Corn	Peaches
Cranberries	Pineapple
Plums	Pumpkin
Prunes (least acid)	Apples
	Watermelon
	Milk
	Peas (least basic)

¹ The terms *acid-ash* and *alkaline-ash* indicate excess of potential inorganic anions and cations, respectively.

bones is not available until circulatory changes induce active catabolism in isolated bone areas.

The ultimate sources of stored base are the involatile cations of the diet. Relative acid-base values of foods are given in Table 7. Physicians seldom consider the absolute quantities of inorganic bases in foods, but they must know which dietary constituents to increase when extra base is required. Fruits, vegetables, and milk are the best dietary sources of base. In general, animal foods and cereals give rise to an acid ash due to the sulfuric and phosphoric radicals of proteins. Cranberries, plums, and prunes are exceptional fruits; they contain appreciable amounts of benzoic and quinic acids which are converted to hippuric acid and excreted in the urine.

Foods contain very little hydroxide, carbonate, bicarbonate, or basic phosphate; but they do contain appreciable amounts of potential base in the form of salts of organic acids. In the animal body, these organic

TABLE 8

APPROXIMATE pH OF FRUITS AND CERTAIN OTHER FOODS

Lemons	2.0
Cranberry juice	2.4
Cola beverages, ginger ale	2.7
Vinegar	3.1
Grapes, pickles, plums	3.2
Grapefruit	3.3
Pineapple, strawberries	3.4
Sauerkraut	3.5
Apples, peaches	3.6
Oranges, cherries	3.7
Honey, prunes	3.8
Pears	4.0
Tomatoes	4.2
Apricots, blackberries	4.4
Karo syrup	4.5
Buttermilk	4.6
Cheese (cream)	4.8
Banana	5.1
Watermelon	5.4
Cucumbers, bread	5.5
Beans (cooked), carrots	5.8
Oysters	5.9
Potatoes (cooked)	6.0
Ham (boiled)	6.2
Oatmeal (cooked)	6.3
Beef (broiled)	6.4
Cantaloupe, cream	6.5
Egg	6.6
Corn (cooked), milk	6.7

anions are separated from the involatile cations by metabolic processes. The natural fruit juices are acid in reaction (Table 8), but their ash is alkaline because they contain buffer systems of organic acids and their salts, as, for example, acetic acid and potassium acetate in vinegar. Organic anions such as acetate, citrate, malate, and tartrate are oxidizable to carbonic acid and water in the body, liberating cations. It is partly because they contain readily available base that lemon and orange juices are freely administered to patients with suspected acid-base disturbances.

The storage of base in tissues and fluids has definite limitations, a discussion of which must be postponed until the relations of base to water have been considered. Usually, the body is in a fairly delicate state of base equilibrium because of storage limitations; the excess base provided in a single day's diet is retained in the body for only a few days. It is evident that our bodies maintain normal acid-base equilibrium only when adequate base is being continually absorbed from the intestinal tract. The proper assimilation and storage of base is dependent on the maintenance of healthy tissues through adequate intake of protein, vitamins, and the trace elements (copper, iodine, iron, etc.). The diet must also have sufficient caloric value, but must not contain excessive quantities of fat.

THE ALKALI RESERVE

This term, often used in discussions of acidosis, signifies the titratable alkalinity of the blood plasma. It represents the alkali available for neutralization of involatile acids, in contradistinction to the true alkalinity or pH. It is actually the pH which has the greatest significance for life and cell function, but accurate determination of plasma pH is attended by difficulties, and the variations found in early disease are not large enough for clinical interpretation. The determination of alkali reserve has greater practical value and its variations parallel many of the clinical signs of acidosis.

The entire alkali reserve, including the alkaline components of all buffer systems, is not usually determined. Estimates of the bicarbonate content of plasma are sufficient as this anion is the most important transport form. Even the plasma bicarbonate estimation is approximated by determination of the total carbon dioxide; the



ratio is only $\frac{1}{10}$ at pH 7.4, and, hence, little error is introduced by including the small fraction of carbonic acid.

Several methods have been proposed for determining the alkali reserve. One method is to add a known quantity of standard hydrochloric acid to the plasma, remove the liberated carbon dioxide by aeration, and titrate the excess hydrochloric acid to the pH of the original plasma (using

neutral red as indicator). An indirect method is the determination of the carbon dioxide content of alveolar air. The normal value of the latter is 5.6 volumes per cent, equivalent to a partial pressure of 40 mm. of mercury. When the pulmonary membranes become abnormal, as in pneumonia and emphysema, the determination of alveolar carbon dioxide is not a true measure of arterial conditions. Hyperpnea, resulting from irritation of the respiratory center or fever, also decreases the alveolar carbon dioxide tension.

The most widely used method for alkali reserve determination is Van Slyke's gasometric procedure, which measures the volume of carbon dioxide gas liberated from plasma after adding excess lactic acid and subjecting the mixture to a partial vacuum in a closed apparatus. A description of this apparatus and the details of the method are given in many laboratory textbooks. The normal alkali reserve of plasma and cerebrospinal fluid, as determined by Van Slyke's method, is in the vicinity of 60 volumes per cent of total carbon dioxide (about 95 per cent of which represents bicarbonate).

Sellards' test is an approximate tolerance test that roughly evaluates the alkali reserve. In this test, 5 gm. doses of sodium bicarbonate are given by mouth every two hours, and the approximate pH of the urine voided at the end of each period is determined. When the urine shows an increase of approximately 0.3 pH, it is assumed that the normal alkali reserve has been re-established in the blood. The amount of bicarbonate given, prior to this result, provides an index to the original deficiency. The method proves the absence of acidosis, but it is neither exact nor reliable in diabetic or nephritic conditions. In the former the excretion of acetone acids, and in the latter the excretion of base, are erratic.

THE AMMONIA MECHANISM

Cells of the distal tubules of the kidney can manufacture a limited amount of ammonia from blood amino acids and glutamine. The cells of the tubular epithelium secrete ammonium bicarbonate which reacts with involatile acids to form other ammonium salts. This renal mechanism is stimulated by a relative deficiency of body base or by an excess of involatile acid. Most herbivorous animals excrete ammonium salts only during starvation; their body proteins are then being actively catabolized, and their normally alkaline urine becomes acid.

From these considerations, one could predict that ammonium salts will increase in the urine of patients with acidosis. During the first few days of acidosis, the involatile acids combine with some of the available stored base of the body, and later the increasing demand for alkali stimulates the ammonia mechanism. The normal urinary excretion of ammonium salts in man is equivalent to approximately 0.7 gm. ammonia daily, but it fluctuates with the diet. Ammonium formation increases

with acid diets or when involatile acids accumulate in the blood, but not in carbonic acid accumulation.

Urinary ammonia decreases during alkalosis and in chronic glomerulonephritis, where kidney function is impaired by degeneration of the kidney tissue. In early acute nephritis and in nephrosis urinary ammonia output is not altered.

Our discussion has centered about urinary ammonium salts. Blood ammonia levels do not participate in these changes because the urinary ammonia is excreted rapidly.

ACID-BASE BALANCE AND FINAL ADJUSTMENT OF NEUTRALITY

Acid-base balance is the term designating the control of body neutrality. It applies not only to the body as a whole, but, in a limited sense, to the control of the electrolyte balance in individual tissues and cells. The total base and total acid of the blood are normally constant even though individual cations or anions may be interchanged. The average normal concentration and distribution of determined cations and anions (as milliequivalents per liter) in human blood plasma are as follows: total base, 154, consisting of sodium, 142; calcium, 5; potassium, 5; and magnesium, 2; and total acid, 154 (chloride, 105; bicarbonate, 25; proteinate, 17; other organic anions, 4; phosphate, 2; and sulfate, 1). In neutralization reactions a milliequivalent (meq.) is equal to 0.001 gram-atom of hydrogen. It is, therefore, equal to a milliliter of N solution.

There are frequent reciprocal changes in plasma bicarbonate and chloride levels, and in erythrocytic diphosphoglycerate¹ and chloride levels which compensate each other and maintain the total anion concentration of the blood, unless abnormal quantities of other anions have intruded, as in ketosis. The disturbance of this balance toward excessive acidity or alkalinity produces pathological disturbances known as acidosis and alkalosis, respectively. Acidosis may be caused either by loss of base, increase of acid, or both. The lungs and kidneys are primarily concerned in preventing such abnormalities; the intestine and skin also assist in slighter degree. The excretory organs can vary their acid-eliminating function, whereby a condition of compensated acidosis is maintained and the symptoms are slight as long as the pH of blood and tissues remains near normal.

EXCRETION BY THE KIDNEY

The kidney excretes acid phosphate, which is the chief factor in determining the pH of urine. This is a much slower process than pulmonary carbon dioxide excretion, and it is less efficient because some base is lost from the body. To change blood filtrate to urine of average composition

¹ Human erythrocytes contain approximately 130 mg. per cent of diphosphoglyceric acid, an important non-diffusible anionic constituent of these cells.

requires that the phosphate buffer ratio be shifted from $\frac{1}{2}$ to $\frac{2}{3}$; the kidney can, on occasion, change it to $\frac{5}{9}$. Diuresis, that is, increased volume of urine, hinders this conversion and may raise the urine pH by 1.0, as more base is lost in the urine. Hence, urines of small volume generally have a lower pH than those of larger volume. The site of phosphate selection is the distal renal tubule. Here cations, chloride, bicarbonate, and about three fourths of the phosphate are reabsorbed through the tubular epithelium into the blood, but carbonic acid is not. The kidney can effect a 40 per cent saving of base by the reaction of carbonic acid with the basic phosphate in the lumen of the tubule: $B_2HPO_4 + H_2CO_3 \longrightarrow BH_2PO_4 + BHCO_3$. The carbonic anhydrase of the renal tubule is probably involved in the reabsorption of bicarbonate. The bicarbonate content of urine varies with the urinary pH, being as much as 10 gm. daily in alkaline urine and practically absent from very acid urine. The urinary loss of base as bicarbonate is thus greatly diminished in acidosis.

The daily urine of a normal adult contains about 185 meq. of anions and 185 meq. of cations. Approximately 80 per cent of the cations are involatile base, the other 20 per cent being ammonium. The anions are 75 per cent inorganic and 25 per cent organic; free acids account for 15 per cent of the total anions. The free titratable acid is determined by titration with 0.1 N alkali, using, as indicator, either neutral red with an end point at pH 7.4 or phenolphthalein with an end point at pH 8.2. Values obtained with neutral red more nearly represent the physiological excretion of acid. Normal urine contains from 100 to 600 ml. of 0.1 N titratable acid, chiefly BH_2PO_4 , per day. A small fraction represents the excretion of unneutralized hippuric, uric, and other organic acids. The excretion of organic acids is increased during ketosis. In blood plasma, the acetone acids exist as salts, but in urine 5 to 55 per cent are free. In Table 9, the combined value of ammonia plus titratable acidity of the urine is shown as an index of "total" acid excretion. This index is a measure of the base economy and alkali reserve in the body. A value in excess of 27 ml. of 0.1 N acid per kg. of body weight indicates acidosis. In nephritis and advanced hepatic disease, this index is unreliable.

RÉSUMÉ

The physiological disposal of acid by the body may be summarized as follows:

1. Body buffers and special neutrality mechanisms speedily react with approximately 1 liter of N acid, thus allowing temporary neutralization and safe transportation to excretory organs.
2. The most rapid excretory process is the disposal of carbon dioxide by the lungs.
3. The kidneys more slowly restore the acid-base balance by excreting

involatile acids and sparing base through the substitution of ammonium cations.

4. A variable portion of certain organic acids is oxidized to carbon dioxide by the tissues.

5. The skin eliminates some lactic acid in the perspiration, and the intestine excretes a portion of the phosphate.

PATHOLOGY

"The wells of rational knowledge offer no magic potion to those who thirst for the absolute certainty, but they do offer us the living waters which strengthen us in our arduous journey."

— MORRIS R. COHEN

ACIDOSIS

Acidosis is a clinical syndrome, that is, a series of related symptoms occurring in a variety of diseases. It is, fundamentally, a disturbance of the acid-base balance which occurs when acid is formed or absorbed faster than it can be destroyed or excreted; or when the loss of body base exceeds the dietary intake. The chief symptoms of acidosis are due to efforts of the body to compensate for the pathological condition. They include:

1. Hyperpnea, or increased depth and rate of respiration to improve ventilation and remove carbon dioxide.

2. Rapid pulse, or tachycardia, related to the circulatory problems.

3. Dehydration, or loss of body water, which accompanies the loss of base. This symptom establishes the important correlation that base and water are transported together in the body. Manifestations of dehydration include dry skin and emaciation.

4. Malaise, headache, and nausea are minor and variable symptoms.

5. Dyspnea, or difficult breathing, occurs in advanced stages of acidosis. It is not complicated by cyanosis (a bluish discoloration of body surfaces due to excessive amounts of reduced hemoglobin) unless anoxic or asphyxial complications are present.

6. Coma, the final symptom, occurs when the alkali reserve is reduced below 20 volumes per cent and the plasma pH falls to 6.9 or 7.0. The cardiac and respiratory centers of the brain collapse because of the abnormal metabolic conditions induced by the low pH. The respiratory and cardiac rates become slow and irregular, and death soon follows unless treatment is instituted.

The alkali reserve may fall to 30 volumes per cent before hyperpnea, headache, and nausea appear. At this time there need be no dyspnea, provided the patient is at rest. In fact, the alkali reserve may fall to approximately 15 volumes per cent before alarming symptoms occur, probably because the clinical determination does not include the total available alkali of the body. Determination of the alkali reserve, there-

fore, detects mild or initial stages of acidosis before they are clinically demonstrable by physical examination (Table 9).

TABLE 9
LABORATORY FINDINGS IN ACIDOSIS AND ALKALOSIS
(METABOLIC TYPES)

	PLASMA ALKALI RESERVE (Vol. % CO ₂)	PLASMA pH	ALVEOLAR CO ₂ TENSION ¹ (Vol. % CO ₂)	SELLARDS' TEST (Grams NaHCO ₃ per 70 Kg.)	URINE ACETONE (Gm. per 24 hrs.)	URINE ml. 0.1 N Acid + NH ₃
Alkalosis	Over 75	7.5-7.8	Over 6.5	0	1.5	
Normal (adult)	55-75 ²	7.4	5.2-5.7 ³	0-30	0.02	0-1500
Acidosis:						
Compensated	35-55	7.3-7.4	3.5-5.0	30-55	5-10	1500-4000
Severe	20-35	7.2-7.3	2.0-3.5	55-75	10-25	4000-6000
Coma	Below 20 ⁴	6.9-7.2	Below 2.0	Over 75	25-150	Over 6000

¹ Marriott method, using rebreathed air.

² 40 to 50 in children.

³ Values approximately one tenth lower in women and children.

⁴ The threshold of coma varies with infections and other complications.

In the hospital laboratory, simple qualitative tests for urinary acetone and acetoacetic acid are used to differentiate ketosis from other possible forms of acidosis. These tests (page 180) are usually confirmatory of ketosis, but they are also positive in the occasional non-acidotic ketonurias accompanying alkalosis and infections.

Types of Acidosis

Clinically, acidosis may be classified as: *metabolic acidosis* in which the alkali reserve is below 50 volumes per cent, and *respiratory acidosis* with a normal or elevated alkali reserve. In metabolic acidosis, non-volatile acids are responsible for the condition; in the respiratory type, carbonic acid is concerned. Respiratory acidosis is not of immediately serious concern because carbon dioxide diffuses through lung tissue into the alveolar air twenty-five times as rapidly as oxygen, and the patient tends to suffer more severely from anoxia. Treatment of respiratory disease is, therefore, directed toward this symptom; in pneumonia, for example, oxygen inhalation is indicated. The alkali reserve undergoes a secondary, compensatory increase in respiratory acidosis, whereas it remains low in the metabolic type. Hence, both alkali reserve and plasma pH decrease in metabolic acidosis, while only the pH falls consistently in the respiratory type.

Acidosis may be either *compensated* or *uncompensated*, depending on the acceleration of functions which counteract the pathological condition and

maintain the pH of the blood near normal; but even in compensated acidosis the alkali reserve is usually low.

Metabolic Types of Acidosis

Ketosis. This is the principal type and the most severe form of metabolic acidosis. It is the result of an accumulation of acetoacetic and β -hydroxybutyric acids in the tissues and tissue fluids incident to diabetes mellitus, starvation, or other conditions of abnormal carbohydrate utilization. Children are especially susceptible to this form of acidosis. In healthy adults, moderate ketosis is initiated during the first few days of starvation. It is mild because carbohydrate utilization is not grossly abnormal. The stored carbohydrate is soon consumed but the body continues to form some carbohydrate from tissue proteins. The acetone acids are neutralized and excreted as salts, thereby causing a continual loss of base from the body. During the first two days of starvation sodium is partially withdrawn from body fluids; as this supply is depleted, potassium loss mounts and atrophy begins in those tissues from which the potassium is being withdrawn. As the tissue proteins are catabolized, their nitrogen and sulfur appear in the urine as ammonia, urea, and sulfate. The alkali reserve may fall as low as 35 volumes per cent during starvation.

Since fasting incites ketosis, this type of acidosis complicates other types and appears in a variety of diseases. Ketosis is observed following anesthesia, during prolonged vomiting, in malnutrition of children, and in the toxic vomiting of pregnancy. In the last-named condition, and also in normal pregnancy, there is a second source of acidosis, namely, the transfer of base from the mother to the fetus. By the ninth month of pregnancy the alkali reserve is approximately 10 per cent below the normal standard, and the plasma pH is lowered by 0.05. In the toxemia of pregnancy known as eclampsia, the plasma pH is 0.15 to 0.2 below normal.

Inasmuch as the precursors of the acetone acids include fats, it is not surprising to find that a high fat diet, that is, a diet in which fat is increased at the expense of other foods, initiates ketosis in human subjects. Clinicians have used such diets to produce acidosis in epileptic patients and in patients with genito-urinary infections. The object of this therapy is to produce antispasmodic effects by lowering the plasma pH and thus to decrease the epileptic seizures; or, in the case of urinary infections, to inhibit bacterial growth by bathing the mucous surfaces with urine of pH 4.8 to 5.5. β -Hydroxybutyric acid is the active bacteriostatic agent below pH 5.5. Ammonium chloride and ammonium mandelate are also administered for this purpose. The ammonium chloride acidifies the urine (page 40), and mandelic acid resembles β -hydroxybutyric acid in its bacteriostatic effects. In recent years, sulfonamide therapy has largely replaced acidifying measures, except for *Streptococcus faecalis* infection

(page 477). Continued administration of sulfanilamide lowers the alkali reserve, inhibits carbonic anhydrase, and increases the sodium excretion.

It has been mentioned that ketonuria appears during alkalosis. Administration of alkali to fasting patients or to those already suffering from ketosis temporarily readjusts the pH of the body fluids and relieves such distressing symptoms as dyspnea. However, alkali therapy encourages subsequent accumulation of the acetone acids, and may cause excessive retention of sodium and massive water storage or edema. Sodium chloride is often preferred for replenishing the plasma base in diabetic patients, since the diabetic is deficient in chloride owing to the prolonged polyuria. The accepted treatment for severe ketosis includes insulin injections and glucose by mouth or intravenously. Insulin is a pancreatic hormone which causes rapid and efficient utilization of carbohydrate and thereby inhibits the abnormal formation of acetone acids. Thiamin (vitamin B₁) and hormones of the adrenal cortex have smaller alleviating effects on the ketosis of starvation.

When ketosis appears in its severest form, as in diabetes mellitus, the diphosphoglycerate concentration of the erythrocytes is lowered, and the blood and tissues may contain as much as 400 mg. per cent of total acetone. The urinary acid excretion may reach 150 ml. of N acid daily in addition to 500 ml. of N ammonium salts. As much as 50 gm. of total acetone and 5 gm. of ammonia nitrogen may be excreted. Since considerable involatile base is lost in the urine as salts, the base and sodium chloride content of the diet are important. Sodium chloride by mouth affords relief from certain annoying symptoms, such as abdominal pain. Excretion of acetone acids is quite irregular, especially when insulin is administered, but ketonuria is usually marked in the late afternoon or evening.

Ketosis can be incited or aggravated by a variety of factors, including anesthesia, infectious processes, liver pathology, shock and physiological disturbance of carbohydrate utilization. Severe lowering of the blood sugar level and hepatic glycogen following insulin overdosage induces ketosis. This type of acidosis leads to marked dehydration, increased protein catabolism, increased cardiac rate, rapid and irregular respiration, depression of the central nervous system, coma, and death. The metabolic origin of acetone acids is considered on page 224.

Acidosis from Lactic Acid. Accumulation of lactic acid produces a mild form of acidosis. Lactic acid accumulates in final stages of liver disease (advanced cirrhosis and acute diffuse necrosis), toxemias of pregnancy, fevers, and such asphyxial conditions as anesthesia, cardiac disease, post-hemorrhagic conditions, pneumonia, tuberculosis, and shock. The blood lactic acid of cardiac patients has been reported as high as 110 mg. per cent. The increased blood pyruvic acid level which accompanies severe cardiac decompensation is unimportant in considerations of acidosis.

Acidosis from Loss of Body Fluid. Diarrhea, vomiting, hemorrhage, and

intestinal or pancreatic fistulae cause an acidosis that is not primarily due to accumulation of acid, but rather to a loss of body fluids and the base contained in them. Diarrhea causes an appreciable loss of sodium, calcium, and potassium through the intestine. The great water loss also aggravates the acidosis, especially in children. The attendant partial or complete starvation induces an accumulation of organic acids and causes a further fall in the alkali reserve.

The low plasma bicarbonate leads to a compensatory increase in plasma chloride, which enters from the tissues. This is an important consideration in the treatment of severe diarrheas, hemorrhage, and so forth. The presence of dehydration suggests that the greatest assistance would be provided by injections of physiologic sodium chloride solution or Ringer's solution. However, the blood bicarbonate is already low, the chloride often is increased, and the introduction of additional sodium chloride may further lower the alkali reserve. Lactate solutions containing sodium and other cations are, therefore, often injected instead of saline. Hartmann's solution is used at times for this purpose; as injected, it contains 0.3 per cent sodium lactate, 0.6 per cent sodium chloride, 0.04 per cent potassium chloride, and 0.02 per cent calcium chloride. Injection of lactate solution avoids a marked increase of blood chloride; the lactate anion is gradually removed by the liver, which converts it into carbohydrate. The remaining cations can hold bicarbonate, increasing the alkali reserve. The choice between saline and lactate solutions is influenced by the functional state of the patient's kidneys. If these organs are capable of normal response to increased fluid administration, they will selectively excrete chloride anions so that a normal alkali reserve and acid-base and water balances are re-established. When kidney function is subnormal, lactate should be more efficacious. The injection of from 5 to 10 per cent glucose solution supplies carbohydrate food, and its oxidation combats secondary ketosis. Glucose injections also supply water but do not provide base.

Prolonged vomiting is another common cause of acidosis, which is due, partly, to loss of base and water in the feebly acid or neutral vomitus, and partly to fasting ketosis. This combined acidosis may become so severe in children as to require injections of both glucose and saline solutions. In adults, whose gastric juice is more acid, vomiting sometimes results in alkalosis; the individual result is dependent chiefly on the acidity of the vomited gastric contents. In any case, sodium chloride and other salts are lost from the body.

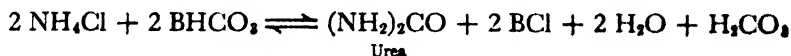
Vomiting is a prominent symptom of gastric and upper intestinal obstructions. Inasmuch as chloride is lost in the gastric vomitus, plasma chloride falls and the erythrocytic diphosphoglycerate and plasma bicarbonate anions rise reciprocally. At times, marked alkalosis and tetany ensue, with alkali reserves as high as 110 volumes per cent. This type of alkalosis, known as "gastric tetany," is accompanied by dehydration and

a loss of base which is partly compensatory. Glucose and saline injections are used in the treatment, since fluid by mouth often aggravates the vomiting. When the obstruction is below the sigmoid, fluid is well absorbed and pathology of acid-base balance is minimized.

Acidosis from Faulty Excretion of Involatile Acid. Acidosis of this type occurs in terminal glomerulonephritis and other destructive kidney lesions. The functional ability of the nephritic kidney is subnormal and such metabolites as acid phosphates, sulfates, and organic acids are not excreted rapidly enough to prevent their accumulation in the body. The ammonia mechanism fails, selectivity in the excretion of cations and anions is decreased, and the concentrating power of the kidney is impaired, leading to acid accumulation and loss of base. The latter is further aggravated by vomiting, diarrhea, starvation, and polyuria.

Despite the converging tendencies, acidosis is a fluctuating complication of nephritis, and it does not accompany general hypofunction of the kidneys when cations are also retained. In the acidosis of nephritis, the respiration is deep and sighing, and tissue wasting is rapid. Treatment of nephritic acidosis is very ineffective because of inability to compensate adequately for loss of kidney function. Occasionally, glucose, saline, or sodium bicarbonate injections are given, depending on the symptoms present. A base-forming diet of fruits and vegetables is provided. Acidosis is usually more pronounced in late glomerulonephritis where it accompanies the syndrome known as uremia; but occasionally it is encountered in severe acute hemorrhagic nephritis. The pronounced edema that frequently develops in these patients is due to sodium and water retention by the kidney; and in this case, acidosis is absent. Both uremia and acidosis can be precipitated by provoking profuse diuresis, which leads to a loss of base.

Therapeutic Acidosis from Acidifying Diuretics. Ammonium and calcium chlorides are frequently employed by physicians to induce acidosis and provoke diuresis. Daily doses of 20 gm. of ammonium chloride or 30 gm. of calcium chloride *per os* produce well developed acidosis in man, and they may lower the alkali reserve as much as 50 per cent. Ammonium salts (chloride, nitrate, or sulfate) produce acidosis as indicated by the equation:



This detoxication reaction occurs in the liver, which converts poisonous ammonium ions into innocuous urea molecules. It is obvious that the alkali reserve is depleted by the reaction, which also has an important side effect, not visible from the equation. Ammonium chloride causes increased production of acetone acids in the normal liver and hence leads to mild ketosis. Sodium chloride excretion is partly responsible for the diuresis, which is enhanced by the diuretic substance, urea. Note that the

ammonia appearing in the urine after ammonium chloride administration is not the original ammonia. Ammonium, calcium, and magnesium lactates have no acidifying effects because lactate anions are either converted to carbohydrate or oxidized to carbon dioxide and water.

Calcium and magnesium salts of highly ionized acids act as acidifying diuretics, provided the salt can be absorbed in the intestine. The chlorides, nitrates, and sulfates act similarly whether fed or injected. The acidifying effect of calcium and magnesium salts is achieved by a physiological separation of the excretory paths for the cation and anion. Approximately two thirds of the dibasic cations are excreted in the feces as carbonates, soaps, and basic phosphates. The involatile anions are excreted in the urine, largely in combination with sodium and other fixed bases of the body; some are neutralized by ammonia in the kidneys. The first effect of acidifying diuretics is to increase the excretion of fixed base, and the second is to stimulate the ammonia mechanism. Administered calcium chloride has a physiological acidifying effect equal to three fourths of its equivalent of hydrochloric acid. Administered acid phosphates are readily excreted as such by the kidneys without marked loss of base or increase in ammonia production. However, the urine becomes more acid because of the excretion of the acid phosphate.

Respiratory Type of Acidosis

This condition is essentially an accumulation of carbon dioxide within tissues and body fluids, which occurs in pulmonary and cardiac diseases, anesthesia, morphine therapy, and other asphyxial states. It differs from previously described types of acidosis in that the alkali reserve is not decreased. The determination of the alkali reserve is, therefore, of little aid in detecting the acidosis. As mentioned previously, carbon dioxide diffuses through lung tissue approximately twenty-five times as rapidly as oxygen, so that fatality in cardiac and pulmonary conditions is primarily determined by oxygen lack. Hence, cyanosis and other anoxic symptoms predominate; the respiratory acidosis is usually compensated and is of secondary clinical importance. Slight exertion lowers the plasma pH approximately 0.1 in cardiac patients.

There is usually less hyperpnea and more dyspnea and headache than in the metabolic types of acidosis. Also, the increase in urinary ammonia is much less noticeable, because the chief involatile acid which accumulates is the lactic acid produced by the partially asphyxiated tissues. Respiratory acidosis does not produce polyuria or dehydration; in fact, the urine flow is usually diminished. Because of the impaired circulation in heart disease, carbon dioxide accumulates mostly in the tissues, and the plasma base and pH tend to be near normal. In pneumonia, the cations and chloride anions of serum and urine are lowered when sodium chloride accumulates in tissue fluids prior to the crisis; after the crisis, the sodium chloride is swept into the blood and is excreted in the urine.

Principles of Treatment of Acidosis

The chief therapeutic measures are the following:

1. Treatment of the disease causing the acidosis, a process requiring time.
2. Replacement of lost base by supplying fruit juices, milk, and vegetables in the diet.
3. Administration of alkaline buffers, such as sodium lactate or sodium bicarbonate, for emergency relief of threatening symptoms.
4. Replacement of lost base and water by injections of physiologic sodium chloride solution.
5. Glucose and insulin injections for combating ketosis.

No set prescription is desirable; the symptoms of each case should be analyzed in terms of physiology and metabolism.

ALKALOSIS

This condition represents a relative accumulation of base in the body. It is comparatively rare inasmuch as the main metabolic processes in the body lead to acidity. Important symptoms of alkalosis are decreased rate of respiration, due to increased pH of the plasma, and hyperirritability of the neuromuscular system, known as *tetany*. This term should not be confused with tetanus bacillus infection.

The early symptoms of tetany, namely, increased reflexes and spastic phenomena, are diagnosed by appropriate neurological tests (page 710). The nervous irritability may develop into muscular twitchings and convulsive states. Tetany also develops as the result of hypofunction of the parathyroid glands, in which case there is no true alkalosis but rather a deficiency of ionized blood calcium. The production of tetany by alkalosis is predicted by *Bödlander's* equation, which expresses the relation between the concentrations of hydrogen and calcium ions in the blood plasma:

$$[\text{Ca}^{++}] = K \frac{[\text{H}^+]}{[\text{HCO}_3^-]}$$

It is actually decreased calcium ion concentration which produces the tetanic state both in hypoparathyroidism and in alkalosis. In the latter, the calcium ion decrease is secondary to the lowered hydrogen ion concentration (page 623).

Metabolic Alkalosis

The metabolic type of alkalosis results from considerable loss of hydrochloric acid in vomitus, as in pyloric stenosis, upper intestinal obstruction, or prolonged gastric lavage. It is also produced by prolonged or excessive administration of alkali in the treatment of peptic ulcer. In fact, most

patients treated for peptic ulcer by the Sippy milk, cream, and sodium bicarbonate regimen have high alkali reserves. Clinical symptoms of alkalosis may develop in these patients, especially when kidney function is inadequate. Much smaller quantities of sodium bicarbonate can produce the same condition in nephritic patients with subnormal excretory function. Deep x-ray, radium, or prolonged ultraviolet irradiation sometimes produces alkalosis. In metabolic alkalosis, the alkali reserve is above 75 volumes per cent; when the plasma pH rises to 7.6, tetany develops. The respiration is slowed and the urinary ammonia decreases. Gastric tetany, caused by vomiting, is the only form of alkalosis in which dehydration occurs. In alkalosis, the kidneys excrete a little extra alkali, but when the sodium cation of blood becomes slightly reduced, this compensatory excretion stops and the urine becomes acid in spite of the alkalosis. Ingestion of alkali causes increased intestinal excretion of calcium and phosphate, and tends to diminish the urine flow.

Respiratory Alkalosis

Alkalosis is an expected result of hysteria, encephalitis, fever, and other states of hyperventilation. Excessive amounts of carbon dioxide are swept from the blood stream and the alkali reserve may fall to 20 volumes per cent. Respiratory alkalosis causes diuresis.

Treatment of alkalosis includes the administration of calcium chloride, ammonium chloride, or hydrochloric acid by mouth, the injection of calcium gluconate, and the inhalation of carbon dioxide. Injection of saline and glucose is suggested if vomiting occurs.

BIBLIOGRAPHY¹

CHEMISTRY

General

- HITCHCOCK, D. I. *Physical Chemistry for Students of Biology and Medicine*. Ed. 3. Springfield, Thomas, 1940.
JOHLIN, J. M. *Introduction to Physical Biochemistry*. New York, Hoeber, 1941.
WEST, E. S. *Physical Chemistry for Students of Biology and Medicine*. New York, Macmillan, 1942.

pH Determination

- BRITTON, H. T. S. *Hydrogen Ions*. Ed. 3. London, Chapman and Hall, 1942.
CLARK, W. M. *The Determination of Hydrogen Ions*. Ed. 3. Baltimore, Williams and Wilkins, 1928.
KOLTHOFF, I. M., and LAITINEN, H. A. *pH and Electro Titration*. Ed. 2. New York, Wiley, 1941. (Methods.)

¹ References of a general nature will be found in the Addendum.

METABOLISM

General

- GAMBLE, J. L. Extracellular Fluid. Boston, Harvard Medical School, 1942.
ROUGHTON, F. J. W. Recent work on carbon dioxide transport by the blood.
Harvey Lect., 39 : 96, 1943-44.
SHOHL, A. T. Mineral Metabolism. New York, Reinhold, 1939.

Foods

- BRIDGES, M. A. Dietetics for the Clinician. Ed. 4. Philadelphia, Lea and Febiger, 1941.

Respiration; Excretion

- GOODMAN, H. The hydrogen ion concentration of the skin. *Urol. & Cutan. Rev.*, 47 : 470, 1943.
HALDANE, J. S., and PRIESTLY, J. G. Respiration. New Haven, Yale Univ. Press, 1935.
KROGH, A. Comparative Physiology of Respiratory Mechanisms. Philadelphia, Univ. Penn. Press, 1940.

PATHOLOGY

Cardiac Disease

- ALTSCHULE, M. O. Pathological physiology of chronic cardiac decompensation. *Medicine*, 17 : 75, 1938.
CHRISTIE, R. V. Dyspnea. *Quart. J. Med.*, 7 : 421, 1938.
HARRISON, T. R. Failure of the Circulation. Ed. 2. Baltimore, Williams and Wilkins, 1939.
WHITE, P. D. Heart Disease. Ed. 3. New York, Macmillan, 1944.

Ketosis

- DARROW, D. C., and CARY, M. K. Nondiabetic ketosis with acidosis. *J. Pediat.*, 6 : 676, 1935.
JOSLIN, E. P., *et al.* The Treatment of Diabetes Mellitus. Ed. 7. Philadelphia, Lea and Febiger, 1940.
MACKAY, E. M. The significance of ketosis. *J. Clin. Endocrinol.*, 3 : 101, 1943.
SOSKIN, S., and LEVINE, R. Physiological and clinical aspects of ketosis. *Am. J. Digest. Dis. & Nutrition*, 11 : 305, 1944.
WALTHER, H. W. E. Urinary antiseptics. *J. A. M. A.*, 109 : 999, 1937.

Miscellaneous

- REIMANN, H. A. The Pneumonias. Philadelphia, Saunders, 1938.

CHAPTER II

COLLOIDS, ENZYMES, AND OXIDATION



COLLOID CHEMISTRY

"The cell is a delicately balanced moving equilibrium, — a complicated system of systems."
— MORRIS R. COHEN

NATURE OF COLLOIDS

The word *colloid* refers to a state of subdivision of matter. True solutions are homogeneous and consist of one phase. A colloidal solution is a two phase system. There is not a sharp line of demarcation between colloids and true solutions but, in general, colloidal particles are larger than molecules and have diameters ranging approximately from one-half micron to one millimicron.¹

The colloidal particles are termed the *dispersed* or *discontinuous phase*, and the medium, the *dispersion medium* or *intermicellar phase*. The term *micelle* is used critically to designate the colloidal particle plus its surrounding electrical double layer. *Eucolloids* are long threadlike micelles of large molecular weight. Both phases of biological colloidal systems are usually liquid in nature, that is, the swollen colloidal particles are dispersed in an aqueous medium.

Colloids pass through ordinary filter paper (pore size, from 2 to 5 microns). During their passage some of the colloidal particles may be retained at the surface of the paper by electrostatic forces. Colloids also pass through the pores of porcelain bacteriological filters which are from 0.2 to 0.6 μ (micron or one thousandth of a mm.) in diameter. However, they are retained by ultrafilters, such as collodion or gelatin gels.

Since colloidal particles are beyond the range of microscopic visibility, their solutions need not be turbid. The particles can be detected by their diffraction images in a powerful beam of light. This phenomenon is termed the "Tyndall effect." It may be observed in the *ultramicroscope*, which is merely a dark-field microscope with provision for intense illumination at a right angle to the visual path. While the number and movements of particles may be investigated, their shape is not revealed. Information concerning the shape and structure of colloidal particles is

¹ One millimicron or 1 $m\mu$ is one millionth of a millimeter.

provided by similar use of roentgen rays for which the individual atoms serve as diffraction points.

The separation of the dispersed phase from the dispersion medium is known as *flocculation* or coagulation, and return to the colloidal state as *peptization*. The stability of a colloidal solution depends on the adsorbed film and the electrostatic repulsion between the particles. Small particles, like small crystals, are more soluble than larger ones. In aging colloidal solutions, the smaller particles go into solution and the larger particles increase in size and tend to flocculate. Peptizing agents decrease the particle size, increase the stability, and prevent or delay the flocculation of colloids. Particle sizes of biological colloids are subject to change during metabolic processes.

SURFACE AREA

The small size of colloidal particles confers on them a great specific surface. A cube with 1 cm. edge has a surface of 6 sq. cm., but after division to colloidal dimensions its surface is approximately 6000 sq. m. or 1.5 acres. Such division produces a tremendous number of particles and transforms internal into external or surface material. The chemical and physical properties of matter in the colloidal state differ from those of gross material in the direction of increased surface activity. The surface molecules of colloidal particles are oriented, allowing certain chemically active radicals to project into the dispersion medium. Protoplasmic constituents are thus oriented at the interfacial films surrounding biological particles. According to the *Gibbs-Thompson rule* substances which lower surface tension concentrate in surface layers, and vice versa. Emulsions, that is, dispersions of liquid particles in liquids, become more stable as the interfacial tension is lowered, and any substance that lowers the surface tension will stabilize the emulsion. This is illustrated by the well known stabilizing effect of soaps on oil in water emulsions. Local changes in surface and interfacial tensions precede the morphological rearrangements of mitosis.

LYOPHILIC AND LYOPHOBIC COLLOIDS

Colloids have been classified as lyophilic and lyophobic according to their ability to attract the solvent,¹ the attraction being greatest in the case of the lyophilic colloids. Most biological colloids are lyophilic, while inorganic colloids are more often lyophobic. The lyophilic colloids have swollen particles that are liquid or semiliquid. In contrast to lyophobic colloids, they tend to foam and to assume viscous jellied forms. The lyophilic colloids require large concentrations of neutral salts for their flocculation; they also show reversible flocculation, whereas lyophobic

¹ In the case of aqueous colloidal solutions the terms *hydrophilic* and *hydrophobic* are used.

colloids are irreversibly flocculated. The only stabilizing factor of lyophobic colloids is their electrostatic charge, while lyophilic colloids are additionally stabilized by solvent shells.

ELECTROKINETIC POTENTIAL

Colloidal solutions will conduct an electric current, although less readily than solutions of ionized electrolytes. Electric charges are present at the surfaces of micelles, and the particles are surrounded by electrical double layers. The surface charge has a threefold origin, namely, contact potential with the intermicellar fluid, true ionization of molecules of the colloid particle, and capture of ions from the surrounding fluid by a process known as adsorption. Colloidal iron, $\text{Fe}(\text{OH})_3$, can form positively charged cations which are flocculated by certain anions; gum mastic shows the opposite behavior.

Proteins, phospholipides, and certain other cell constituents are amphoteric colloids whose particle charges depend upon the pH of the environment. Hence, animal tissues can be stained with either acid or basic dyes by merely shifting the pH of the dye bath. The pH at which an amphoteric system ionizes equally as acid and base is termed the *isoelectric point*. Here, the particles are electrically neutral and proteins apparently exist as neutral, doubly charged, *dipolar ions* or *zwitter ions*. At the isoelectric point a protein colloid shows minimal viscosity, swelling, and solubility, and maximal flocculation. The electrokinetic potential does not have to become zero before colloids flocculate; there is a critical zone near the isoelectric point at which the interfacial charge is so reduced that it no longer stabilizes the colloid. A list of isoelectric points is given in Table 10.

TABLE 10
APPROXIMATE ISOELECTRIC POINTS

	pH		pH
Alanine	6.0	Aspartylhistidine	4.9
β -Alanine	6.9	Aspartyltyrosine	2.85
Alanylalanine	5.8	Avidin	10.0
Alanylglycine	5.5	Bee venom	8.7
Alanylproline	5.7	Bence-Jones protein	5.2
Amylase (malt)	4.4	Biotin	3.25
Anserine	8.3	Bushy stunt virus	4.1
Arachin	5.15	Canaline	6.5
Arginase	6.6	Canavanine	7.9
Arginine	10.8	Carbonic anhydrase	5.3
Asclepain	3.1	Carbonylhemoglobin	6.65
Asparagine	5.4	Carboxylase	5.1
Aspartic acid	2.8	Carnosine	8.2
Aspartylaspartic acid	3.0	Casein	4.6
Aspartylglycine	3.3	Catalase	5.4

TABLE 10 (Cont.)

APPROXIMATE ISOELECTRIC POINTS

	pH		pH
Chlorocruorin	4.5	Glycyltyrosine	5.7
Collagen	7.7	Glycylvaline	5.7
Complement, endpiece	6.35	Gonadotropin (pregnancy urine)	3.3
Complement, midpiece	5.3	Gonadotropin (pregnancy mare serum)	2.6
Conarachin	3.95	Growth hormone	6.85
Corticotropin (sheep)	4.7	Hemerythrin	5.8
Cozymase I	3.1	Hemocyanins	4.7-6.3
Crotalus venom	7.9	Hemoglobin	6.8
Crotoxin	4.7	Histidine	7.6
α -Crystallin	5.1	Histidylglycine	6.8
β -Crystallin	6.1	Histidylhistidine	7.3
Cysteine	5.1	Histone (thymus)	12.0
Cysteinylcysteine	5.0	β -Hydroxyglutamic acid	3.3
Cystine	5.0	Hydroxyproline	5.8
Cytochrome <i>c</i>	10.65	Hypertensin (angiotonin)	6.4
Diiodotyrosine	4.3	Influenza virus (PR8)	5.3
Diphtheria antitoxin	7.0	Insulin	5.35
Diphtheria toxin	4.1	Isoleucine	6.0
Edestin	5.7	Keratins	3.4-4.8
Elastin	6.9	Lactalbumin	4.7
Erythrocytes (human)	6.7	β -Lactoglobulin	5.2
Euglobulin (serum)	5.7	Lecithin	6.4
Ferritin	5.4	Leucine	6.0
Fetuin	5.0	Livetin	4.9
Fibrin	6.6	Luteinizing hormone (pig)	7.45
Fibrinogen	5.4	Luteinizing hormone (sheep)	4.6
Fibroin (silk)	2.2	Lysine	9.7
Follicle-stimulating hormone	4.8	Lysozyme	10.8
Gastroglobulin	3.5	Lysylglutamic acid	6.1
Gelatin	4.8	Lysyllysine	10.0
Gliadin	6.5	Methemoglobin	7.9
Globin (human)	7.5	Methionine	5.7
Globin (denatured)	8.2	Mucoid (vitreous humor)	3.5
Globulin X	5.2	Myoalbumin	3.3
Glutamic acid	3.2	Myogen	6.7
Glutamine	5.65	Myoglobin	7.0
Glutathione	2.8	Myosin	5.5
Glutelin	6.4	Norleucine	6.1
Glycine	6.1	Nucleic acid	0.7
Glycinin	5.0	Nucleoprotein (pancreatic)	3.5
Glycylalanine	5.7	Nucleohistone (thymus)	4.0
Glycylaspartic acid	3.6	Nucleosides	5.4-8.2
Glycylglycine	5.6	Nucleotides	1.5-4.5
Glycylleucine	5.7	Ornithine	9.7
Glycylproline	5.7		

TABLE 10 (Cont.)

APPROXIMATE ISOELECTRIC POINTS

	pH		pH
Ovalbumin	4.85	Sarcosine	6.1
Ovomucoid	2.8	Scarlet fever toxin	5.5
Ovoverdin	6.7	Secalin	6.7
Oxyhemoglobin	6.65	Secretin	7.5
Papain	9.0	Serine	6.0
Paraperoxidase	10.5	Serum albumin	4.8
Parathormone	4.8	Serum albumin (denatured)	5.4
Penicillin B	4.4	Serum globulin, α	5.05
Pepsin	2.75	Serum globulin, β	5.1
Pepsin inhibitor	3.7	Serum globulin, γ	6.0
Pepsinogen	3.8	Sphingomyelin	6.0
Phenylalanine	5.5	Sulfomucins	2.7-4.5
Phenylalanylarginine	10.0	Taurine	5.1
Phycocyan	4.85	Thiamin	9.5
Phycocerythrin	4.25	Threonine	5.6
Pitocin	8.5	Thromboplastin (beef lung)	5.1
Pitressin	10.8	Thyroglobulin	4.55
Pituitrin	4.8	Tobacco mosaic virus	3.2
Pneumococcus antibody (horse)	4.8	Trypsin	7.5
Pneumococcus antibody (rabbit)	6.6	Tryptophane	5.9
Poliomyelitis virus	6.0	Tuberculin (bovine)	4.3
Prolactin	5.7	Tyrosine	5.7
Proline	6.3	Tyrosylarginine	8.5
Protamines	9.7-12.2	Tyrosyltyrosine	5.6
Protamine insulin	7.3	Urease	5.0
Prothrombin	5.0	Valine	6.0
Pseudoglobulin (serum)	5.0-6.3	Verdoperoxidase	10.0
Rennin	4.6	Yellow enzyme	5.2
Rhodopsin (visual purple)	4.5	Zein	6.7
Ribonuclease	8.0	Zymohexase	6.3
Salmin	12.0		

Passage of micro-organisms and colloids through Berkefeld and Chamberland bacteriological filters depends not only on the size but also on the electrostatic charge of the particles. Certain infectious agents which pass bacteriological filters have been named *filtrable viruses*. The common bacteriological filters are essentially porous silica with a negative surface charge which allows negatively charged particles to pass, if these are small enough, but retains and adsorbs positively charged particles. Conversely, filters of gypsum have a positive charge and allow the passage of positively charged particles. The classification of infectious agents as filtrable viruses had to be corrected for such electrostatic phenomena. It has been shown that at least certain filtrable viruses are not living cells but are large complex molecules of nucleoprotein (page 492).

ACTION OF ELECTROLYTES

Peptizing agents often stabilize colloidal solutions by affecting the electrostatic charge on the particles; and, conversely, electrolytes flocculate lyophobic colloids by neutralizing the interfacial charge. The active ion of the salt flocculant is the one whose charge is opposite in sign to that of the colloidal particles. The valence of the ion is important; the flocculating power of trivalent ions is over a thousand times that of

TABLE 11

HOFMEISTER'S LYOTROPIC SERIES
(In Order of Decreasing Effect on Proteins)^{1,2}

ANIONS (Most Effective in Acid Solution)	CATIONS (Most Effective in Alkaline Solution)
<i>Polyvalent</i>	<i>Monovalent</i>
Citrate	Lithium
Tartrate	Sodium
Sulfate	Potassium
Phosphate	Rubidium
<i>Monovalent</i>	Caesium
Acetate	Ammonium
Fluoride	Hydrogen
Chloride	<i>Polyvalent</i>
Bromide	Magnesium
Nitrate	Calcium
Chlorate	Strontium
Iodide	Barium
Thiocyanate	Heavy metals

¹ For effects on peptization and imbibition of colloids — read up on left and down on right. For salting out — read down, but for flocculation by cations in alkaline solution — read up. For hydration of ions — read down.

² The indicated valency relations are constant but the order of individual ions within a valence group varies in specific cases because, in the last analysis, the Hofmeister series is based on chemical activities.

monovalent ions. For this reason, ferric and aluminum salts are used to flocculate colloidal organic impurities from drinking water supplies. Even isovalent ions vary in their flocculating power and can be arranged in a series known as the *Hofmeister* or *lyotropic series*. The relations of this series to colloidal solutions are summarized in Table 11. Such lyotropic effects are appreciable only in concentrated salt solutions.

Dilute electrolytes are not efficient flocculants for lyophilic colloids because the lyophilic particles are stabilized by hydrated shells. To rob the micelles of this water of hydration requires the use of dehydrating substances. When concentrated salt solutions are employed for this purpose, the process is termed "salting out." Similar dehydration can be accomplished by alcohol. Partial dehydration of hydrophilic colloids at

their isoelectric points, or other methods of very slow flocculation, can cause separation of the solution into two liquid phases — a phenomenon known as *coacervation*.

GELS

A *gel* is the more rigid form of a lyophilic system; the less rigid form is termed a *sol*. Sols may be converted into gels by (1) lowering the temperature (gelation of gelatin); (2) changing pH (flocculation of casein from milk); (3) adding electrolytes; (4) dialyzing out a peptizing agent; (5) allowing a desiccated lyophilic colloid to absorb fluid; or (6) changing the solvents (preparation of collodion gel from ether and alcohol solution). Sol-gel transformations are important life phenomena; a well known example is the clotting of blood. Certain gels are temporarily liquefied by mere shaking, a phenomenon known as *thixotropy*. Thixotropy has been shown to occur in living cells; it represents a change in the orientation of micelles.

In gels, the greatly swollen micelles form a nearly continuous structural pattern which greatly increases the rigidity and water-binding power. The micelles are arranged as a fibrillar framework whose interlacing fibrils entrap fluid by capillary forces. The micelles in gelatin gels consist of long polypeptide chains interlinked at their midportions, but with loose ends which can join with adjacent micelles. The sol-gel transformation is usually reversible, although very slight chemical changes in proteins cause irreversible gelation. Such gels contain only small proportions of the original intermicellar fluid and separate as coagula. Histological fixing methods transform sols into gels and coagula, and they often change colloids from lyophilic to lyophobic systems. Stable gels tend to become anisotropic or doubly refractive when mechanically strained or deformed.

Biological sols and gels represent different arrangements of the same micelles in varying degrees of hydration. The outer zone of a hydrophilic micelle contains molecular layers of water of hydration, sometimes termed "bound water." In the outer zones of the micelles the molecules of the intermicellar fluid are closely packed and specially oriented. When much fluid is held in such fashion a contraction of volume occurs. Gels have been prepared with an internal pressure sufficient to cause them to explode violently. The hydration of colloidal particles increases as the particle size is decreased. The tendency to gelate is proportional to the affinity between micellar and intermicellar phases.

In a number of sols the phenomenon of *double refraction* appears on stirring or flowing, owing to the fact that even in sols the colloidal fibrils are oriented. Double refraction is often seen in biological mixtures as, for example, in virus preparations, mitotic spindles, muscle cells, and amoebae. Many protein molecules appear to be slender fibrils that can combine to form macromolecular aggregates or particles with enormous molecular

weights (in the case of viruses, 400,000 to 8,500,000,000). The contractility of certain cellular materials, such as muscle proteins, has been found, by x-ray studies, to be due to shortening, folding, or helical contraction of the ultramicroscopic protein fibrils. Energy for these changes is supplied by oxidations in the cell.

ADSORPTION

Concentration of materials from the dispersion medium on the surfaces of colloidal particles is called adsorption. During this process dissolved salts, organic compounds, ions, and also other colloids of the intermicellar phase are concentrated at the interface. When ions are adsorbed, the potential at the interface is altered. Adsorption compounds are not constant in composition because the usual valence forces of all molecules in the colloidal particle are not involved. Adsorption is more than mere mechanical concentration, since chemical combination can take place through accessory or residual valences. It is a rapid process whose equilibrium is affected by the concentration of the material being adsorbed, the specific surface of the colloidal particles, etc.

Adsorbed substances are often held firmly and do not diffuse readily, because they have undergone chemical changes at the interfacial surfaces. Ovalbumin seeks gas-water interfaces, and when its solutions are shaken this protein accumulates in the foam where it is chemically changed to insoluble denatured albumin. This is an example of *pseudo-adsorption* or adsorption followed by an irreversible chemical change. The protein enzyme, trypsin, is adsorbed on charcoal and may then be eluted from its adsorption compound by shaking with casein solution. A second adsorption compound is preferentially formed between the trypsin and casein.

The more highly hydrated a substance is, the less readily it is adsorbed and the more readily it aids the adsorption of other less hydrated substances. For example, potassium and sulfate are highly hydrated ions, sufficient concentrations of which drive proteins into surface layers and cause flocculation. The relative hydration of ions follows the Hofmeister series (Table 11, page 50). Since adsorption is a surface phenomenon, its rate is increased by rising temperature, but the amount of material adsorbed is thereby decreased. Surface manifestations usually have negative temperature coefficients.

Adsorption leads to local accumulation of soluble materials in living tissues. Nuclear seeding also favors local deposition in cells, since small particles add to larger ones in their vicinity. Rates of diffusion and points of entrance into cells are determining factors in the formation and extension of histological precipitates. Dyes and other colloidal reagents tend to flocculate outside the cells when they cannot readily diffuse into them.

PROTECTION

The term *protection* signifies the stabilization of a colloid against the action of flocculating agents. Colloidal solutions frequently consist of three components: the dispersed phase, the dispersion medium, and a second dispersed lyophilic colloid which protects the first from flocculation. Lyophilic colloids are very excellent protective agents. In bile, the difficultly soluble substances, cholesterol and calcium bilirubinate, are protected from flocculation by soaps, bile salts, and proteins. Also, lyophilic proteins and phospholipides hold the fatty substances of living cells in an invisible colloidal form. Proteins, lecithin, sphingomyelin, glycogen, higher homologues of soaps, monovalent cations (except protons), and most anions tend to disperse fats and oils in water. The substances which stabilize water in oil systems include cholesterol, cholesteryl esters, cephalin, cerebroside, divalent and trivalent cations, and hydrogen ions. Note that potassium ions act like lecithin and that both have a vagotonic action in the body, while calcium ions and cholesterol are sympathotonic. When the normal protective mechanisms of the cell fail, the more insoluble substances such as fats and cholesterol flocculate into microscopically visible droplets and particles. Such pathological changes are found in fatty degeneration and calculus formation. There is a close correlation between decreasing liver glycogen and the appearance of fatty degeneration in hepatic cells.

The protective action of lyophilic colloids is measured by the *Zsigmondy gold number*, which is the concentration of colloid necessary to stabilize a standard gold colloid against flocculation by standard sodium chloride solution. A decreasing order of protection (increasing gold number) has been established for gelatin, casein, gum arabic, ovalbumin, sodium oleate, and starch. Gelatin is several thousand times as effective as starch.

OSMOTIC PRESSURE AND DIFFUSION

Colloidal solutions have a measurable osmotic pressure, but it is comparatively slight because of the relatively small number of particles. The osmotic pressure of colloids, termed *oncotic pressure*, is very important in connection with water transport in the body, Donnan membrane equilibria, and so forth. The oncotic pressure of human blood plasma colloids is approximately 28 mm. of mercury.

When diffusible substances penetrate into gels, they may produce complex precipitation and crystallization patterns. The most widely studied phenomena of this kind are the *Liesegang rings* or rhythmic precipitation bands (spirals in stretched gels) seen in natural agates and in cross sections of biliary and urinary calculi. Such diffusion phenomena provide a possible basis for the formation of symmetrical chemical structures in living colloidal material.

IMBIBITION

Many lyophilic colloids swell in aqueous solution; even crystalline proteins do so. This process, known as imbibition, is rapid and liberates heat. The imbibition pressure, or pressure against which a colloid can swell, is sometimes as high as 20,000 atmospheres. It is much greater than ordinary osmotic forces. Certain gels are very efficient dehydrating agents; at 18° C. 1 gm. of gelatin can retain 27 gm. of water, and at 15° C. a 13 per cent gelatin gel resists a pressure of 200 kg. per sq. inch without the separation of fluid. A 3 per cent micellar continuum binds the 97 per cent of water in a jellyfish in a form rigid enough to withstand the weight of an adult man. Aging gels may contract spontaneously to squeeze out some of their water of imbibition. This process, termed *syneresis*, causes the exudation of serum from clotted blood, vitreous humor, and so on. Glandular secretion involves imbibition of tissue fluid by cells, followed by syneresis into the glandular lumen.

During imbibition the colloidal particles behave as though they were surrounded by semipermeable membranes. The swelling of colloids is influenced by ions in the order given in the Hofmeister series. Carbohydrate gels are most swollen at pH 7, while many proteins show maximal imbibition in the vicinity of pH 3.0 and 10.5, conditions that are far removed from the pH of living tissues. Contraction of muscle is due not to swelling of the protein micelles, but rather to a change in their shape (page 386).

MEMBRANES

Biological membranes, such as intestinal, ectodermal, and renal epithelia, are often polar in type. Their irreciprocal permeability (*i.e.*, passage of solutes in only one direction) can be reproduced *in vitro* in gelatin gels. Irreciprocal permeability is an expected property of layered or double membranes. The unilateral location of enzymes within cells can induce functional polarity in the transfer of certain substances. One should not picture living epithelia as membranes whose polar permeability is conditioned by structure alone. Living cells are never in a state of passive equilibrium with their surrounding fluid; instead, they maintain a "steady state" which is dependent on energy derived from metabolism. Hence, metabolic processes affect permeability. This is well illustrated by the permeability of the epithelium of the proximal renal tubule to phenol red (phenolsulfonphthalein). The epithelial cells normally excrete the dye from the blood to the urine, but when cell oxidation is inhibited the polar permeability ceases.

The passage of molecules is not always through the pores, meshes, or intermicellar phases of membranes. Cell surfaces are mosaics of proteins and lipides. Certain substances can permeate these micelles. Water-soluble constituents penetrate protein and phospholipide micelles, while

fat-soluble substances enter fat and steride micelles. The fat-soluble dye, Sudan III, stains butter but does not stain milk. The continuous phase of butter consists of fat which has been flocculated from colloidal emulsion in the milk by removal of the protecting colloid, casein. In fatty degeneration, the cell lipides become stainable after the protection by proteins and phospholipides has been disturbed. Fat stains, therefore, give an indication of the state of the colloidal system but do not detect the highly dispersed lipide. Sharp zones in fixed or stained histological specimens are not necessarily preformed membranes, since the Liesegang effects produce such zones.

COLLOID METABOLISM AND PATHOLOGY

"Instead of accepting experience, science discriminates between the experience of truth and the experience of illusion." — MORRIS R. COHEN

A systematic discussion of colloid metabolism would not, as yet, be profitable. The fundamental physiological implications are not always clear, and metabolic processes are inseparably connected with most biological colloidal manifestations. Important specific colloidal phenomena related to the absorption of foods from the digestive tract, water transport, and immunity will be discussed in the chapters devoted to these subjects. At this time we shall consider the general colloidal relations of blood, capillaries, and tissue fluids; and the pathological phenomena of inflammation, hemolysis, blood clotting, and calculus formation.

BLOOD PLASMA COLLOIDS

Blood plasma colloids are chiefly protein and lipoprotein sols; only at the surface of the erythrocyte is the gel state approached. An adult person has approximately 2700 ml. of circulating blood plasma (55.5 ± 6 per cent of the whole blood), with a protein content of 190 gm. — equal to that of the entire liver. The blood plasma may, therefore, be considered as being a colloidal organ whose particle surface is approximately 35 acres, as compared with 0.8 acre for the erythrocyte surface. Normal human plasma contains 7.0 ± 1 per cent of protein, consisting chiefly of albumin (4.5 per cent), globulin (2.25 per cent), and fibrinogen (0.3 per cent). The preservation of normal plasma protein levels is an important function of the liver, which can replace plasma proteins lost through acute hemorrhage. Normal kidneys aid in maintaining the concentration of plasma proteins, while nephrotic kidneys allow plasma proteins to escape into the urine. When the normal plasma protein concentration is lowered by liver disease, abnormal transformations within the body, or by excretion through diseased kidneys, serious consequences follow (page 443). A sudden loss of circulating plasma protein, even without simultaneous depletion of erythrocytes, induces severe circulatory shock.

VEHICULAR FUNCTIONS OF PLASMA PROTEINS

The capillaries of a man's muscles, if laid end to end, would reach two and one-half times around the earth. Certain transported crystalloids remain in the blood traversing these permeable capillary networks and are eventually delivered to, and removed by, specific tissues. This requires a more selective mechanism than mere diffusion. The plasma protein colloids act as adsorption vehicles for many soluble food substances, metabolic products, salts, water, and pigments. The assimilation of these substances by certain tissues depends partly upon their ability specifically to separate the adsorbed material from plasma proteins.

The vehicular effects of plasma proteins can be demonstrated in gelatin gels, which do not allow the passage of Congo red particles until after they have been adsorbed on plasma proteins. Such passage of adsorption compounds through membranes is termed the *embatic effect*. Dyes of varied particle sizes lose their specific charges and diffuse through gels at almost equal rates when adsorbed on plasma albumin. Bile salts and other hydrotropic substances (page 149) can displace these adsorbed materials from plasma proteins and allow their accelerated passage into adjacent tissues. The liver secretes bile salts; therefore, this organ specifically removes intravenously injected Congo red and excretes it in the bile. In nephrotic patients, whose plasma proteins are abnormal and depleted, adsorption of the dye is disturbed. Congo red then leaves the circulation abnormally by various paths, one of which is excretion in the urine with the plasma albumin.

The bile pigment, bilirubin, is also adsorbed on plasma albumin and is liberated from it by bile salts in the liver. When blood bilirubin is increased above a certain concentration, or when bile salts enter the blood from the liver (as in obstructive jaundice), the kidney can also excrete bilirubin. If this pigment is injected intravenously into nephrotic patients, the decreased plasma albumin no longer provides for specific excretion by the liver, and bilirubin rapidly leaves the blood to enter other tissues. Other substances known to be partly or wholly adsorbed on plasma albumin are: calcium ions, Congo red, digitalis glycosides, eosin, melanogen, salicylic acid, sulfonamides, uric acid, and urobilinogen.

Carotene, cholesterol, phospholipides, Sudan III, and thorotrast are adsorbed chiefly on plasma globulins. For this reason, appreciable amounts of plasma cholesterol and lecithin are not ether extractable. Adsorbed cholesterol is liberated by bile salts in the liver. The placenta and certain other tissues can liberate Sudan III from its globulin adsorption compound. In nephrotic and xanthomatous patients, there are parallel increases of plasma cholesterol and globulin. The increase in plasma globulin in many infectious diseases may also have embatic significance. Consideration of the embatic effect suggests that the clinical value of blood transfusions is not limited to the provision of erythrocytes;

plasma protein vehicles are also furnished, and these contribute to the beneficial results.

ERYTHROCYTES

Erythrocyte membranes may be pictured as equilibrated mosaics of lipides adsorbed on a protein gel matrix. The permeability of these membranes is conditioned by the relative amounts of cholesterol and lecithin in the dimolecular lipid layers at the surfaces. Studies with isotopes have shown that sodium and potassium cations do not penetrate mammalian erythrocyte membranes readily. Under normal conditions, the membrane is about fifty thousand times more permeable to anions than to cations, and more permeable to Cl^- and HCO_3^- than to PO_4^{---} and SO_4^{--} . However, at pH 8.2 the membrane allows cations to pass more freely than anions. This demonstrates the significance of the membrane potential. The similar electronegative potentials of erythrocyte and capillary surfaces allow free circulation of the blood, and prevent occlusion of the capillary lumen. Capillary membranes differ from erythrocyte membranes in that they allow the passage of cations, plasma proteins, and free hemoglobin. While erythrocytes are not penetrated by plasma proteins, the latter are adsorbed on them and contribute to the behavior of the erythrocyte membranes. Leukocytes are similarly affected by plasma proteins; they exhibit ameboid movements only in colloidal media, and their migration is influenced by the pH of the medium. At pH 7.0, leukocytes migrate in all directions, while at pH 6.3, they move toward acid regions such as inflamed tissues.

Sedimentation Rate

The sedimentation rate of erythrocytes is a non-specific clinical test, useful in studies of tuberculosis, rheumatic fever, and rheumatoid arthritis, in differentiating gynecological lesions, and in prognosticating infections and anemias. In acute infections the rate tends to increase about one to two days after the body temperature and leukocyte count rise. Moderate acceleration of sedimentation occurs in the blood of patients having cardiac decompensation, jaundice, leukemia, lymphoma, malignant neoplasms, nephritis, thrombosis, or traumatic injury. The increased sedimentation rates (exceeding 10 mm. at one hour for males, or 20 mm. for females) found in these conditions are often traceable to an elevated globulin and fibrinogen content of the plasma. Elevation of serum albumin lowers the sedimentation rate. The ratio of cholesterol to phospholipide in the plasma also affects sedimentation; cholesterol and its esters, which form hydrophobic sols, accelerate sedimentation while hydrophilic lecithin sols retard it.

Hemolysis

The passage of hemoglobin out of the erythrocytes into the plasma is termed hemolysis. While there are obvious relations to osmotic pressure,

hemolysis through purely osmotic forces is unknown. Membrane changes always occur, and the preservation of normal erythrocyte membranes depends on the maintenance of their dispersed phospholipide (lecithin) and cholesterol mosaic. The normal mosaic is most stable at osmotic pressures represented by 0.73 to 0.82 per cent sodium chloride solutions. Hypotonicity (or hypertonicity) beyond these limits tends to destroy the mosaic. The addition of cholesterol to hypotonic solutions protects erythrocytes against hemolysis, while phospholipide added to isotonic solutions is hemolytic.

Human erythrocytes vary in their resistance to hemolysis; their fragility increases in aging blood banks. The resistance of erythrocytes to a graded series of hypotonic sodium chloride solutions forms the basis of the *fragility test* of Rous. Normally, from 0.42 to 0.44 per cent sodium chloride initiates hemolysis, and 0.32 to 0.34 per cent sodium chloride renders it complete. In chronic hemolytic jaundice and certain purpuras, the erythrocytes become fragile, so that even 0.9 per cent sodium chloride may induce hemolysis. Erythrocytes generally become spherical when undergoing hemolysis in hypotonic plasma; the circulating erythrocytes of hemolytic jaundice patients are spherical. The fragility of erythrocytes in the various anemias is discussed on pages 550 to 553.

Saponins cause hemolysis by combining with the cholesterol of the membrane. Bile salts are also quickly adsorbed on the erythrocyte membrane, where they lower the surface tension and destroy the membrane by peptizing its lipides. Other fat-soluble substances such as alcohols, soaps, fatty acids, and narcotics act similarly. These are all adsorption hemolysins.

The hemolytic action of certain bacterial toxins, snake venoms, and the poisons of stinging insects is more complicated. It is true that the substance in cobra venom responsible for hemolysis is adsorbed on the erythrocytes and that cholesterol antagonizes its action, but this hemolysin is an enzyme (a lecithinase) which hydrolyzes the phospholipides of the erythrocyte membrane to form the very hemolytic substances, lysolecithin and lysocephalin (page 189). Toxins of streptococci, staphylococci, tetanus bacilli, malaria and scarlet fever organisms, and more virulent strains of smallpox and diphtheria are especially hemolytic.

The injection of foreign erythrocytes causes specific hemolytic antibodies to appear in plasma. Lysis of foreign erythrocytes by these hemolysins requires the participation of the so-called complement of plasma. Complement is a complex globulin-phospholipide colloidal system that is adsorbed on the foreign erythrocyte membrane and induces primary changes preparatory to the action of the specific hemolysins. The latter then act on some membrane constituent to produce hemolytic substances. These immune reactions are considered in detail on page 465.

Hemoglobin is normally confined to the erythrocytes, but *in vivo* hemolysis allows the pigment to enter the plasma (hemoglobinemia).

Such pronounced hemolysis as occurs in severe malaria (blackwater fever) produces sudden massive hemoglobinemia, disturbs the normal colloid equilibria, and causes chills, fever, and malaise. The paroxysmal hemoglobinuria of syphilis is caused by hemolysins which are activated by exposure to cold. Erythrocytes from such patients can be hemolyzed *in vitro* by cooling the blood and then bringing it back to body temperature. This is termed the *Donath-Landsteiner phenomenon*. (See also page 551.)

CAPILLARY PERMEABILITY

Capillaries are subject not only to nervous influences, but also to the colloid equilibria of the blood plasma and tissue fluids with which they are in contact. These thin walled passages do not have an interfering mantle of muscle, hence they allow rapid interchanges between the plasma and tissue fluids. Only a few crystalloids, such as urea and glucose, can diffuse freely through the capillaries. The diffusion of the sodium ion, which favors water retention and edema in tissues, is a partial function of the colloids of the cells and tissue fluids (Donnan equilibrium).

Ophthalmologists have made extensive studies of the permeability of the uveal capillaries.¹ Here, the passage of acid dyes through the capillaries into the intraocular fluid can be observed *in situ*. Basic dyes are usually adsorbed very rapidly at the cell surfaces and are removed from the blood plasma faster than acid dyes, but they do not enter the tissue fluid. These observations demonstrate the electronegative charge on the capillary ultrafilters. The electrostatic charge is also important in secretory processes; only electropositive dyes are secreted in gastric juice, and electronegative dyes in pancreatic juice.

Asphyxiated capillaries become very permeable and allow even basic dyes to pass. Sodium and hydrogen ions, acetylcholine, physostigmine, leukotaxine, purine diuretics, and parasympathetic stimulation increase capillary permeability; calcium ions, adrenaline, atropine, ergotamine, and sympathetic stimulation decrease it. Adrenaline, acetylcholine, and atropine act on the embryonic vascular system before its innervation develops. In fact, the activities of the sympathetic and parasympathetic nerves are mediated by the hormones, adrenaline (or sympathin) and acetylcholine, respectively. The permeability of the capillaries of the uterus and vagina is increased by estrogens. Changes in capillary permeability are not always the result of direct action on the capillary surfaces; at times they are indirect, the result of changes in the plasma colloids. The action of atropine, which increases dilatation but decreases

¹ The transparent structures of the eye are complex gels which show the Tyndall effect. The slitlamp of the ophthalmologist makes use of this effect, and is used to detect swellings and other colloidal changes in the cornea and lens. When the colloids of the ocular tissues and fluids swell, the intraocular pressure increases and glaucoma results. In cataract of the lens there is a degenerative replacement of the normal lens globulin (crystallin) by an insoluble pathological albuminoid.

permeability, shows that capillary permeability is not always proportional to capillary dilatation.

Normal capillaries can undergo changes in permeability to the protein colloids of the plasma. At times they also allow erythrocytes and leukocytes to pass. In asphyxiation, the permeability of the capillaries to plasma proteins may increase fourfold within a few minutes. If oxygen is supplied, the original selective permeability is restored. Collodion membranes, ordinarily impermeable to hemoglobin, are permeated by this protein when surface-active substances such as bile salts are present; the original impermeability is restored when the bile salts are washed out. Damaged tissues liberate substances which act upon capillaries, increasing their permeability to plasma proteins, erythrocytes, and leukocytes. The cellular elements of the blood do not pass through pre-existing openings in the capillary walls, and pores are not observable after the leukocytes have passed. The cells permeate the intact wall by a process termed *diapedesis*. It is in this fashion that erythrocytes penetrate the capillaries of inflamed tissues. Diapedesis occurs during a momentary change of the capillary gel to a sol, a process which resembles the gel-sol change of thixotropy. In a similar manner, droplets of mercury penetrate gelatin gels without producing holes. The formation of the sol form of intercellular capillary substance during leukocyte penetration may, perhaps, be due to the action of surface-active substances on the leukocyte membranes.

LYMPH AND TISSUE FLUIDS

Tissue lymph and pericardial, peritoneal, pleural, and synovial fluids are primarily dialyzates or transudates of blood plasma. The mesenteric chylous lymph which originates in the gastro-intestinal tissues contains, in addition, absorbed digestion products. Many crystalloids such as salts, sugars, and amino acids can freely enter the lymph. The lymphatic endothelia are also partially permeable to proteins, especially to albumins and γ -globulins. The lymphatics and capillaries of the liver are exceptionally permeable to plasma proteins. Capillaries are not as easily permeated by foreign colloids. In traumatic shock, the plasma proteins are decreased by passage into the tissue fluids. The intravenous injection of gum arabic was formerly recommended as substitution therapy; but this procedure is not entirely innocuous. At autopsy, the foreign colloid has been found as plugs in the liver capillaries. The injection of preserved plasma or of lyophile plasma concentrate is more efficient and less toxic, and it has now largely replaced foreign colloid therapy in the treatment of shock.

Since most tissue fluids contain less colloid than does blood plasma, there is a Donnan membrane equilibrium at the walls of the capillaries. As a result, tissue fluids contain slightly greater concentrations of diffusible anions and lower concentrations of diffusible cations than does blood

plasma (Table 100, page 582). Non-ionized crystalloids, such as glucose, urea, and the non-ionizable portion of minerals, are not subject to the Donnan effect. Approximately one half of the plasma calcium is present as a non-ionizable protein complex.

Cerebrospinal fluid is not a mere transudate or ultrafiltrate; its composition is modified by the selective secretory activity of the double (two-cell) epithelial membrane of the choroid plexus. This fluid contains less glucose, urea, uric acid, creatine, iodine, and inorganic phosphate than does blood plasma. There is similar evidence that intraocular fluid is partly secretory in nature.

INFLAMMATION

Inflammation is a pathological metabolic process in tissues. It involves circulatory disturbance; abnormal transfer of cells, colloids, and fluid from blood to tissue fluid; fibrin formation; and cell destruction with subsequent proliferation. While inflammation is one of the physical manifestations of infectious processes it is also a general reaction of tissues to injury, since it can be incited by the injection of sterile distilled water. The condition progresses from a barely visible hyperemia or capillary dilatation to intense suppuration. The increased transfer of fluid to inflamed tissue is due primarily to an increased permeability of the local capillaries and lymphatics, attributable to a substance liberated by the injured tissue. This substance, termed leukotaxine, has been crystallized from inflammatory exudates. It is a dialyzable protein digestion product, or lower polypeptide, active in a dilution of 1:100,000. The capillary-dilating action of leukotaxine is inhibited by adrenal cortical extract. Leukotaxine, certain amino acids, nucleic acids, and foreign proteins are chemotactic for leukocytes, especially for the polymorphonuclear cells. The latter are attracted toward the injured tissue during the early stages of inflammation, at which time the pH of the inflammatory exudate is near 7.2 (about 0.15 pH lower than simple blood transudates and normal tissue fluids). The leukocytosis which accompanies inflammatory processes is apparently due to a pseudoglobulin, the leukocytosis-provoking factor, that appears in inflammatory exudates. It stimulates the release of immature leukocytes from the bone marrow. The euglobulin fraction (necrosin) of the exudate can incite or aggravate the inflammation.

A local acidosis develops in the inflamed area owing to accumulation of carbon dioxide, lactic acid, and other acid metabolites; and the local acidosis increases in proportion to the degree of inflammation. When the pH of the inflammatory exudate falls to about 6.7, the polymorphonuclear cells are injured and are replaced by macrophages. In acute inflammation, the pH of the inflamed area continues to fall to 6.2 or less. This causes degeneration of the macrophages and marked pus formation (suppuration). The protein buffers of the cells and intercellular matrix

are unable to prevent the fall in pH and the resultant cellular destruction within the central inflammatory zone. Paralleling this local acidosis and cellular damage, the potassium of the pus-containing exudate increases from the normal transudate level of 14 mg. per cent to as much as 200 mg. per cent. Spodograms, or ashed histological sections, of inflamed skin show that calcium and magnesium accumulate at the periphery of the inflamed area. This distribution represents a Liesegang zone between the inflammatory center and its less acid environment. The tissue fluid of slightly inflamed areas is like normal transudate in that its chloride is higher and its sodium lower than that of blood plasma. Severe inflammation causes extensive damage to the neighboring capillary walls, and allows the sodium of the tissue fluid to increase while the chloride falls.

There is evidence that gluconeogenesis (conversion of tissue proteins to glucose) occurs in inflamed tissue. The increased catabolism in an inflamed area produces many small molecules from large colloids, increases the local osmotic pressure, and produces an "osmotic storm" of diffusion.

TABLE 12

APPROXIMATE PROTEIN CONTENT OF BODY FLUIDS

	<i>Per Cent</i>
Blood plasma	7.5
Cerebrospinal fluid, aqueous humor	0.025 ¹
Transudates	Low ²
Ascitic fluid, pleural effusions	0.2-5.5
Edema fluid, anasarca fluid	0.1-1.0
Hydrocele fluid	3.5-6.0
Lymph (cardiac)	1.9
Lymph (extremities)	2.0-3.0
Lymph (hepatic)	6.0-8.0
Lymph (intestinal)	4.0-6.0
Lymph (joint) ³	3.5
Lymph (pulmonary)	3.7
Lymph (thoracic duct)	2.0-5.0
Pericardial fluid	2.0
Synovial fluid	1.0-2.0
Exudates ⁴	High
Inflammatory exudates	0.1-5.0
Pus exudates	5.5-7.5
Lymphatic cysts	6.5

¹ As high as 1.0 in disease.

² Simple transudates other than hepatic, intestinal, and thoracic duct lymph usually do not contain more than 2 per cent of protein, but pathological effusions which have existed for some time may become concentrated as the result of the increased capillary permeability accompanying cardiac failure, etc.

³ During activity.

⁴ Blister fluid and angioneurotic edema fluid have high protein concentrations.

Hydrogen and potassium ions are increased in the injured area and can, by their action on autonomic nerves, participate in the local capillary dilatation. Leukotaxine and other nitrogenous products from injured cells incite dilatation and increased capillary permeability, regardless of the pH. The increased hydrogen ion concentration causes the colloids of the plasma, connective tissue, and capillaries to swell and the permeability of the latter to increase. As a result, inflammatory exudates contain more plasma protein than do simple transudates (Table 12). The principal protein of transudates and exudates is serum albumin; but exudates also contain considerable serum globulin, appreciable quantities of fibrinogen, and a mucoid protein of tissue origin. Exudates also have a higher specific gravity and a greater tendency to coagulate than do transudates, and they contain micro-organisms and pus cells.

The water-binding ability of inflamed tissue is markedly increased by the passage of plasma proteins into the tissue spaces. The neighboring capillaries become permeable to erythrocytes, leukocytes, and intravenously injected Congo red and vital dyes, which become localized in the inflamed area. A similar fixation of colloidal irritants is very important in the localization of infections. The early formation of fibrin in inflamed tissues is due to the clotting of plasma fibrinogen which has entered the tissue fluid. This fibrin forms a network about the lymphatics and occludes them. Specific plasma globulins (antibodies) also pass into the inflamed area, where they assist in flocculating foreign proteins and polysaccharides. When foreign proteins are injected into inflamed areas they are poorly absorbed into the circulation.

PERMEABILITY OF THE PLACENTAL BARRIER

The human placenta shows many of the peculiarities of double membranes. Approximately 11 miles of chorionic villi project from the fetal side of the human placenta into the maternal blood and provide a contact area of approximately 12 square meters for diffusion. The human placenta allows the passage of more substances than do the placentae of herbivorous animals and pigs, since in these animals the placenta has added cellular layers. Because of the greater permeability of the human placenta, the mesonephros has less excretory work to perform and the human allantois is correspondingly smaller. Colostrum is relatively unimportant for the nourishment of the newborn infant; the human fetus receives adequate supplies of vitamins, antibodies, and iodine from the mother's blood. The maternal and fetal circulations traverse the placenta in opposite directions, thereby maintaining fetal blood levels near those of the arterial blood of the mother.

In general, small diffusible molecules, such as oxygen, carbon dioxide, chloroform, and certain electrolytes pass the placenta more readily than do larger ones. Experiments with heavy water show that twice its weight

of water is transferred to and from the guinea pig fetus hourly, with complete turnover of the water in the amniotic fluid. The transfer of sodium ion is about fifty times slower. Penetration of the placenta by dyes is in the same order as their passage through gelatin gels. Bacteria do not enter the placenta until the chorionic epithelium has been damaged by toxins; but certain toxins, viruses, and antitoxins penetrate the normal placental membrane. Other toxins, antigens, erythrocytes, and peptones do not pass. The immunity of the human fetus is largely passive in nature, and is partly traceable to antibodies diffusing from the maternal circulation. Certain toxins which pass the placenta may give rise to an active immunity (page 482).

The chorionic epithelium appears to be impermeable to several substances that readily penetrate the capillary endothelia. Such hormones as gonadotropins, insulin, and parathormone¹ do not pass the human placenta easily, while acetylcholine, adrenaline, androgens, estrogens, pitressin, thyroxine, and the thyrotropic hormone do. Vitamins A, D, and E pass with greater difficulty than K and the water-soluble vitamins. Sulfonamides and thiouracil are also transferred readily. Studies with injected glucose, radioactive iron salts, radioactive inorganic phosphate, sodium chloride, urea, uric acid, creatine, and creatinine demonstrate that diffusion of these blood constituents takes place rapidly in both directions. Fetal blood levels of easily diffusible substances fluctuate with the maternal levels. More sugar, fat, phospholipide, cholesterol, carotene, vitamin A, and plasma protein are found on the maternal side; while amino acids, ascorbic acid, bilirubin, citric acid, lactic acid, estrone, glutathione, hemoglobin, non-protein nitrogen, calcium, and inorganic phosphate preponderate in the fetal blood. Fat-soluble substances pass the placenta more slowly than do water-soluble ones. The maternal and fetal blood fat levels are less coordinated than the corresponding blood sugar and non-protein nitrogen levels.

BLOOD CLOTTING

The principal chemical reaction in blood clotting is the conversion of the protein sol, *fibrinogen*, into insoluble sticky *fibrin* gels which arrest hemorrhage by plugging the bleeding areas. Clots formed *in vivo* inside the blood vessels produce a fibrin meshwork that often extends to the nearest point of anastomosis. In rapidly stirred or whipped blood, the fibrin shreds cling to the stirrer and are easily removed from the defibrinated blood. An enzyme, *thrombin*, is necessary for the rapid transformation of fibrinogen to fibrin. Thrombin is present in the circulating blood as an inactive zymogen called *prothrombin*. This zymogen is converted into active thrombin in a preliminary reaction which requires an activator termed *thrombokinas*e. The latter is liberated by injured tissues and, par-

¹ In this text parathormone is used as a synonym for parathyroid hormone.

ticularly in mammals, by the blood platelets or thrombocytes. Thrombokinase, in conjunction with calcium ions, converts prothrombin into active thrombin. The reactions are outlined in Table 13, together with numerous synonyms for the clotting factors discussed below.

Prothrombin

This heat-coagulable glycoprotein is found in the euglobulin fraction of blood plasma; it is gradually destroyed in conserved blood (blood banks). The relative prothrombin concentration is estimated from the prothrombin clotting time (the clotting time of plasma which has been fortified with excess thrombokinase). The average prothrombin clotting time of normal adult blood is 20 ± 2 seconds by Quick's method (15 to 20 seconds by the bedside method). A prothrombin clotting time of 30 seconds indicates a prothrombin level which is 25 per cent of normal, and a tendency to hemorrhage. Patients with a prothrombin clotting time of 40 seconds (10 per cent of the normal prothrombin) are definitely hemorrhagic.

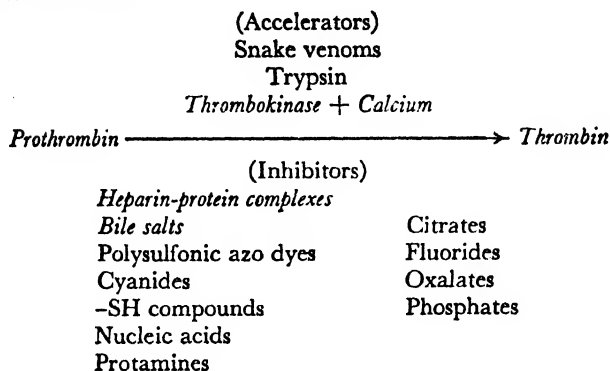
Prothrombin is synthesized by the normal liver when adequate quantities of vitamin K are available. The latter exists in two forms, vitamin K₁ from plants and vitamin K₂ from bacteria, whose chemistry is discussed on page 208. A proposed unit of the vitamin is approximately equal to 3.3 γ of vitamin K₁, 4.25 γ of vitamin K₂, 2 γ of 2-methyl-1,4-naphthohydroquinone diacetate or sodium 2-methyl-1,4-naphthohydroquinone diphosphate, and 1 γ of 2-methyl-1,4-naphthoquinone or 2-methyl-4-amino-1-naphthol hydrochloride.¹ Green plant tissues and certain vegetable and cereal oils are important dietary sources of vitamin K; significant quantities are also manufactured by intestinal bacteria (especially *Esch. coli*). Dietary deficiency of vitamin K induces severe hemorrhagic disease in chickens; in adult mammals the synthesis by intestinal bacteria is sufficiently rapid to compensate for such deficiency. Approximately 75 per cent of infants develop hypoprothrombinemia within 2 to 4 days after birth; the average plasma prothrombin level of the newborn infant is about 60 to 75 per cent of the maternal concentration. It falls to approximately 20 per cent by the second day and frequently remains below 40 per cent during the first five days of life. Hemorrhage from hypoprothrombinemia occurs in 0.5 per cent of newborn infants; the hypoprothrombinemia is more serious and prolonged in the premature. Ingestion of food and water gradually establishes the intestinal flora and incites bacterial synthesis of vitamin K. Daily administration of the vitamin or of 1 mg. of 2-methyl-1,4-naphthoquinone to the mother prior to delivery increases the plasma prothrombin, decreases the clotting time, and offsets hemorrhagic tendencies in the offspring. Parenteral methyl-naphthoquinone therapy is effective if it is instituted at least 4 hours prior to delivery. Marked reduction of plasma prothrombin accompanies icterus neon-

¹ See Table 104, page 646. The symbol γ indicates 1 microgram, or 0.001 mg.

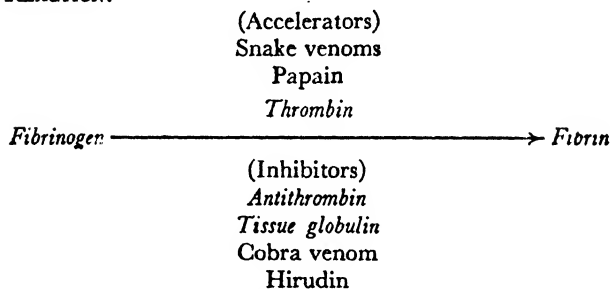
TABLE 13

A. RELATIONS OF BLOOD CLOTTING FACTORS

PRELIMINARY REACTION:



CLOTTING REACTION:



(The principal physiological factors are italicized.)

B. DISTRIBUTION AND SYNONYMS OF CLOTTING FACTORS

IN PLASMA	IN CELLS AND PLATELETS	IN CLOTTED BLOOD
<i>Prothrombin</i>	<i>Thrombokinase</i>	<i>Thrombin</i>
Prothrombase	Cephalin	Fibrinogenase I
Serozyme	Cytozyme	Thrombase
Thrombinogen	Thrombase	<i>Fibrin</i>
Thrombogen	Thromboplastin	
<i>Heparin</i>	Thrombozyme	
Antiprotease		
Antithrombokinase		
<i>Calcium ions</i>		
<i>Antithrombin</i>		
Antithrombase		
<i>Fibrinogen</i>		

torum, anemia neonatorum, and hydrops congenita. Hyperprothrombinemia occurs at times in pulmonary infarction, and following the administration of methylpurines.

The hemorrhagic diathesis induced by obstructive jaundice, bile fistulae, or extensive intestinal diseases, such as chronic ulcerative colitis, celiac disease, multiple polypi, and sprue, is attributable to low plasma prothrombin and to vitamin K deficit. Lack of bile in the intestine prevents the absorption of vitamin K. Since storage of this substance is very limited, the plasma prothrombin may become dangerously low within a few weeks. In the absence of severe damage to the hepatic parenchyma, oral administration of vitamin K and bile salts promptly relieves the bleeding tendency. When the liver can no longer synthesize prothrombin, as in cirrhosis, carcinoma, Banti's disease, and severe hepatitis, vitamin K therapy is of little value, except to confirm the presence of severe hepatic injury. The hypoprothrombinemia which accompanies relapse in pernicious anemia is not affected by vitamin K, but it does respond to liver extract therapy. Massive aspirin, salicylate, or arsenical therapy can cause hypoprothrombinemia in patients.

Daily administration of sufficient vitamin K₁ or of 2-methyl-1,4-naphthoquinone (either orally in oil or intramuscularly in saline suspension or oily solution) gives a maximal prothrombin response within a week. Water-soluble preparations, such as 2-methyl-4-amino-1-naphthol hydrochloride, are sometimes preferred for clinical use. The vitamin is administered as a prophylactic measure before and after biliary surgery, even when the prothrombin clotting time is normal. In emergencies, transfusion of fresh blood provides a prothrombin supply for from six to twelve hours. (See page 672 for further discussion of vitamin K.)

The hypoprothrombinemia which occurs in the hemorrhagic sweet clover disease of animals is caused by the ingestion of *dicumarol* or 3,3'-methylenebis(4-hydroxycoumarin). Administration of this substance to human beings prolongs the clotting time. The effect is counteracted by methylpurines, but not readily by vitamin K; transfusion with fresh blood or injection of prothrombin is temporarily effective. Dicumarol has no effect *in vitro* on the prothrombin time or the clotting time. It exhibits a latent period of approximately twenty-four hours in its action, whether given orally or parenterally. The effect of a single 600 mg. dose continues for six days. Dicumarol therapy diminishes embolism and thrombophlebitis in patients, for whom the suggested doses are 5 mg. per kg. the first day, and 1.5 mg. per kg. on subsequent days. It is important to determine the prothrombin clotting time frequently. Daily parenteral doses of 1 to 2 mg. per kg. in rabbits, and oral doses of 5 to 50 mg. per kg. in dogs, are fatal within a few weeks; they produce hemorrhage, pulmonary edema, and central hepatic necrosis. Dicumarol therapy is contraindicated in endocarditis, and in hepatic or renal disease.

Thrombokinase

This accelerator of thrombin formation is a specific protein-cephalin compound which unites with prothrombin, in the presence of calcium ions. (The chemistry of cephalin and other phospholipides is discussed on pages 187 to 192.) Emulsions of free cephalin, when injected, do not clot blood *in vivo*; also fat-solvent extracts of tissues, which contain free cephalin, have less thromboplastic activity than do the cephalin-protein complexes of aqueous extracts. The latter lose some of their activity when they are digested with the protein-hydrolyzing enzyme, trypsin, or with the cephalin-hydrolyzing enzymes, lipases and lecithinases.

The blood platelets or thrombocytes contain much thrombokinase; they initiate blood clotting by undergoing lysis and liberating the active cephalin complex. Trypsin can liberate thrombokinase from platelets, but this pancreatic enzyme is not concerned in physiological clotting. The blood of normal human adults contains about $350,000 \pm 100,000$ platelets per cu. mm. During the first year of life the average is nearer 250,000. A diminished platelet count (thrombocytopenia) is found in acute myelogenous leukemia, pernicious anemia, purpura haemorrhagica, and destructive diseases of the bone marrow. Thrombocytosis, or increased platelet count, occurs in cachexia, chlorosis, chronic myelogenous leukemia, Hodgkin's disease, and polycythemia vera.

Calcium

Thrombokinase is inactive in dialyzed serum; and the clotting of blood is prevented by precipitating the calcium as oxalate or phosphate or by transforming calcium ions into non-ionized complexes by the addition of fluorides or citrates. While oxalates and citrates have such anticoagulant effects *in vitro*, they have been shown to decrease clotting time when small quantities are injected into animals. From 0.1 to 0.2 per cent lithium or potassium oxalate is commonly used as anticoagulant for analytical blood specimens. Isotonic sodium citrate is used in blood transfusions. Despite the necessity of calcium ions for normal clotting, lowered blood calcium is a very rare cause of bleeding in patients. Strontium can replace calcium in blood clotting; magnesium salts tend to act as anticoagulants.

Heparins and Other Antithrombokinase Factors

Normal plasma contains an antithromboplastic substance which disappears rapidly when in contact with blood platelets in glass vessels. This antithrombokinase shows increased activity in hemophilic plasma.

Heparins, prepared from liver, lung, intestine, kidney, and muscle, are powerful anticoagulants which inhibit the formation of thrombin. The heparins are polysulfuric esters of complex carbohydrates and lipides; those extracted from the tissues mentioned above are mucoitin polysul-

furic acids. Heparins resemble immune haptens (page 469) in their ability to combine readily with plasma proteins through acid radicals. Such anticoagulant heparin-protein compounds appear in blood during anaphylactic and peptone shock. The heparins combine with prothrombin and they dissociate thromboplastins. To exert their anticoagulant action, heparins require a complementary factor in the serum albumin fraction. Basic proteins (protamines) and the basic dye, toluidine blue, rapidly combine with heparins and inactivate them by preventing their reaction with plasma proteins. Protamines can be used, therefore, to determine the concentration of heparin. There is very little heparin in normal blood, but it increases markedly during anaphylactic and peptone shock. Heparin has its origin in the mast cells of loose connective tissue, which are in close association with the vascular system. It is destroyed by the hepatic enzyme, heparinase.

As little as 1 mg. of heparin prevents the clotting of 100 ml. of blood. Heparin preparations are useful in transfusions, in experimental surgery, in the prevention of postoperative adhesions, embolism and thrombophlebitis, and in decreasing fibrin formation in the exudates resulting from bacterial endocarditis, coronary thrombosis, and similar conditions. Heparin, given *per os*, is not absorbed. It is most effective when administered intravenously as a saline solution of the barium salt, 1 mg. of which contains 500 heparin units. The anticoagulant action of heparin is only temporary *in vivo* but with frequent injections the clotting time can be maintained with safety at approximately fifteen minutes. The dosage required for maintenance of a uniform clotting time is quite variable in different patients. Heparin is excreted in the urine.

Bile salts (taurocholates), agar, synthetic polysulfuric esters of polysaccharides, and such polysulfonate azo dyes as Chicago blue, chlorazol fast pink, trypan blue, and trypan red have heparin-like anticoagulant activity. Cyanides and cysteine inhibit clotting by reducing plasma proteins to inactive forms. Nucleic acids and their hydrolytic products are also anticoagulants. The basic protamines can form insoluble compounds with cephalin and thus inhibit blood clotting.

Thrombin

This active plasma enzyme is a low molecular glycoprotein of the euglobulin fraction of plasma which is destroyed by heating above 50° C. It is produced from prothrombin by a chemical reaction accelerated by thrombokinase and calcium ions. The latter combine with prothrombin, but are not integral parts of the thrombin molecule. When purified thrombin is added to blood, it coagulates almost instantly; such preparations are superior to thrombokinase (thromboplastins) as clotting agents for application to oozing surfaces of surgical and hemophilic patients. Dehydrated thrombin preparations are made from prothrombin by means

of thromboplastin obtained from human placenta; the thrombin preparations are more stable than prothrombin *in vitro*.

Antithrombic Factors

Normal blood plasma contains an albumin, *antithrombin*, which specifically inhibits the activity of thrombin. This substance is an enzyme which destroys thrombin rapidly and prevents its excessive accumulation during clotting. Normal blood requires several minutes to clot inasmuch as the thrombin concentration increases only gradually, reaching a peak in approximately one-half hour. Thrombin destruction by antithrombin is so rapid in clotting blood that the average thrombin lifespan is only twenty-four seconds.

Cobra venom and hirudin, from the salivary gland of the leech, have antithrombic effects. Conversely, a number of snake venoms (*Bothrops atrox*, *Bothrops jararaca*, *Notichis scutata*) and staphylococcal extracts cause clotting (Table 13, page 66). Hirudin is directly antithrombic both *in vitro* and *in vivo*. Peptones are anticoagulant only *in vivo*, and they act by stimulating the secretion of hepatic heparin, which not only acts as an antithrombokinase, but combines with a serum albumin to form an antithrombic complex. Certain tissue globulins are antithrombic. By accelerating thrombin formation, excess thrombokinase tends to overcome the anticoagulant effects of hirudin, tissue globulins, and heparins. The various plasma proteins apparently compete for the cephalin and heparin present.

Fibrinogen

This protein is an unstable plasma globulin whose colloidal micelles are fibrillar in nature. Fibrinogen can be determined by diluting the plasma with sodium chloride solution and adding calcium chloride. The fibrin which forms is separated and weighed, or its nitrogen content can be determined. Normal plasma contains 0.3 ± 0.1 per cent of fibrinogen. Plasma fibrinogen is maintained by the liver and decreases when this organ is removed or damaged, as in severe hepatic disease. In hepatectomized dogs, one half of the plasma fibrinogen disappears within twenty hours, indicating that fibrinogen is utilized within the body and must be replaced continually. Diets rich in the biologically valuable proteins of meat and milk cause temporary increases in plasma fibrinogen. High fibrinogen values also occur during menstruation, pregnancy, nephrosis, acute stages of hepatitis, and in infections which cause marked leukocytosis (erysipelas, pneumonia, septicemia, etc.).

Considerable fibrinogen can be removed from an animal by withdrawing blood, defibrinating it, and reinjecting the defibrinated blood. Such losses are replaced by normal animals within five or six hours, provided the liver is intact. Injury or inflammation of tissues is the most powerful stimulus to fibrinogen production. Bacterial products are not the stimu-

lants, inasmuch as sterile and aseptic inflammatory processes are equally effective. Inflammation causes increased utilization of blood fibrinogen in the formation of fibrous exudate.

Fibrin

Fibrin is an insoluble protein split product, a protean, which is derived from fibrinogen by enzymatic hydrolysis. The micelles of fibrin swell tremendously and are insoluble, except in concentrated urea solutions. Thrombin is the normal catalyst for the formation of fibrin; but non-specific proteolytic enzymes, such as papain, and the enzymes of certain snake venoms (page 70) can replace thrombin as catalyzers of this reaction. Crystalline trypsin cannot substitute for thrombin, although it can activate thrombokinase. Fibrin is digested to soluble protein split products by such proteolytic enzymes as pepsin, trypsin, and the fibrinolysin of streptococci. Normal plasma contains small quantities of a fibrinolytic enzyme.

As the fibrin gel ages it undergoes *syneresis* with separation of serum. Retraction of the clot is influenced by constituents in the clotted blood and also by neighboring surfaces. Thus, clots retract from glass but not from amber or paraffin; air surfaces accelerate both clotting and syneresis. Retraction is more rapid in the small blood vessels because of their proportionately great surface areas. Slight differences in blood vessel surfaces determine the direction of retraction and the morphological pattern of the contracted clot. It is common medical knowledge that blood with a high platelet count clots rapidly and forms serum quickly. When the platelet count is less than 45,000 per cu. mm., syneresis is slow or absent, a condition responsible for the soft clots in purpura haemorrhagica.

A number of fibrin products are useful in the clinic. Fibrin clots prepared *in situ* from solutions of purified thrombin and fibrinogen are valuable adjuncts to surgery, skin grafting, and pyelolithotomy. Similarly prepared fibrin foams are excellent hemostatic agents for capillary oozing or venous bleeding. Fibrin films are employed in the treatment of burns, and as dura substitute in neurosurgery. Fibrin fibers, tubes, and plastics have special surgical uses. These products are digested in the body by proteolytic enzymes; the fibrin films can be modified so that they are digested and absorbed within five days, or during longer periods, as desired.

Thrombosis

Thrombi, clotted masses in blood vessels, are important practical problems of medicine and surgery. They occur frequently after operations, in obstetrical cases, infections, circulatory insufficiency, and in cachexia. In these conditions plasma globulin and fibrinogen are often increased. Thrombosis has at least three causes: (1) injury to blood vessel walls, as in atheromatous, sclerosed, infected, or inflamed vessels; (2) slow-

ing of the blood stream, as in heart disease, postoperative conditions, and varicose veins; and (3) changes in the fluidity and composition of the blood which lead to agglutination of erythrocytes and platelets, as after transfusion with incompatible blood. In marked thrombosis, all of these factors are usually involved. The relation of the blood platelets to thrombosis is well established; that portion of the thrombus which is first deposited contains many platelets and leukocytes and is termed a *white thrombus*. The latter extends into the blood stream, entraps erythrocytes, and becomes a *red thrombus*. A thrombus may entirely obstruct the lumen of a blood vessel, but during subsequent syneresis the clot often retracts symmetrically toward the vessel wall and allows the re-establishment of circulation through a central serous channel. This recanalization of the vessel leads to physiological disposal of the thrombus, with little danger of embolism.

Embolism

An *embolus* usually is a detached thrombus, carried by the circulating blood from its original site and impacted in some remote artery. The most frequent cause of embolism is the equal and rapid retraction of an embolus from the entire circumference of the blood vessel. Emboli in the heart, lungs, brain, and other areas where the anastomosing vessels are inadequate to maintain nutrition of the tissues often cause paralysis, gangrene, and death. Embolism in the coronary artery is especially serious and frequently fatal. Isolation of a tissue area from its arterial blood supply by thrombosis or by embolism is called *infarction*.

It is chiefly in the large veins that the thrombi subsequent to abdominal operations, childbirth, and so forth are freed by syneresis before they can be organized by endothelial cells of the blood vessel wall. If the thrombus remains attached and effectively blocks the leg vessels, the limb may swell and varicose veins may develop. When the inflammation of the venous walls is due to infection of the vessels, the thrombosis and inflammation may spread along the veins and cause phlebitis. In surgical patients, emboli often appear suddenly a week or more after the operation while the patient apparently is recovering. Surgeons employ the following measures to prevent postoperative thrombosis and embolism: early movements of the limbs to aid venous circulation, prevention of hindrance to respiratory movements, administration of ample fluid to prevent dehydration and increased blood viscosity, occasional administration of thyroid to accelerate metabolism and circulation, and either dicumarol or heparin therapy.

Clotting Time and Bleeding Time

The normal clotting time of shed blood (average, 4 minutes) may be altered by disease, and it may be shortened by processes which increase the adrenaline level of the blood. Certain patients, "bleeders," suffer

from the inherited condition of *hemophilia*, in which the clotting time may be as long as one hour. These patients continue to bleed from even very small wounds, and the persistent loss of blood leads to anemia. Hemophilia is most frequent in men; in fact, true hemophilia is inherited as a sex-linked recessive character, being transmitted through females and becoming manifest in males. The euglobulin fraction of normal plasma proteins contains a substance capable of accelerating the clotting of hemophilic blood. The blood platelets of hemophilic patients are not readily disintegrated, and they liberate thrombokinase very slowly. It is probable that the thrombokinase of the hemophiliac is abnormal, and increased antithrombokinase activity has been detected. Corrective measures employed in treating hemophilia include blood transfusions and the application of thrombin preparations, snake venoms, or tissue extracts at the bleeding points. The delayed clotting of jaundice is due to low plasma prothrombin (page 67). This condition is a serious complication in gall-bladder surgery.

In *purpura haemorrhagica*, also termed thrombocytopenic purpura or Werlhof's disease, the blood platelets are decreased below 60,000 per cu. mm., and hemorrhage occurs through the tissue capillaries. The clotting time is normal but the bleeding time (normally 2.5 minutes) is greatly prolonged. The soft clots which are formed do not readily undergo syneresis. Splenectomy often restores the platelet count and affords relief. Administration of the amino acid, methionine, also assists restoration of normal clot retraction in thrombocytopenic purpura. In the dietary disease, scurvy, capillary bleeding occurs in the mucous membranes, kidneys, and joints. This is a non-thrombocytopenic purpura, symptomatic of capillary damage (page 667). Similar subcutaneous or submucous hemorrhages are encountered in diphtheria, leukemia, scarlet fever, streptococcal infections, smallpox, and certain anemias and drug intoxications. The occasional benefit of calcium therapy in such conditions may be due to its effects on the capillaries.

Menstrual blood is incoagulable; since it contains neither fibrinogen nor thrombin, it may be regarded as blood which has already clotted.

URINARY CALCULI

The urinary concretions most frequently encountered contain the difficultly soluble urinary components listed in Table 14. Less frequently, urinary calculi consist of cystine, xanthine, bacteria, fibrin, indigo, or lipides. The urinary concentration of salts and the pH of the urine are prime factors in determining the solubility of potential calculus components.

Increased urinary ammonia favors the formation of magnesium ammonium phosphate; but when this ammonia is due to acidosis, the low urinary pH increases the solubility of the salt and effectively counteracts

the ammonia. However, when the ammonia originates from bacterial hydrolysis of urea, as in bladder infections, the pH rises and the urine is greatly supersaturated with the salt. At pH 8.4, the urine will contain approximately eleven times the saturating concentration of magnesium ammonium phosphate and seventeen times that of tricalcium phosphate and of calcium carbonate.

In *phosphaturia*, the secondary calcium phosphate of the urine may be eight times saturated at pH 7. In leukemia, the urinary output of uric

TABLE 14

SOLUBILITY RELATIONS OF DIFFICULTLY SOLUBLE URINE CONSTITUENTS AT AVERAGE CONCENTRATIONS

CALCULUS-FORMING SALT	URINE IS SUPERSATURATED
Secondary calcium phosphate	Above pH 5.3
Magnesium ammonium phosphate	Above pH 6.2
Uric acid	Below pH 6.0
Sodium or ammonium acid urate	pH 4.6-9.0
Calcium oxalate	Above pH 4.0
Acetylsulfonamides	Below pH 7.0

acid increases and, if the urine is scanty, it may contain thirty-five times the saturating concentration of uric acid. Purine or meat diets increase the uric acid output. The concentrated acid urine which at times results from acidosis may contain fourteen times the saturating concentration of uric acid.

Control of the pH of the urine by varying the acid-base content of the diet, or by giving buffer salts or acidifying diuretics, is of some value in preventing urinary calculi, but will not resolve those already formed. Calculi are essentially insoluble *in vivo*; they contain an irreversibly flocculated protein matrix which is resistant to urinary enzymes and to leukocytes.

Increased urinary concentrations of salts are usually determined by two factors, namely, increased excretion of the salt and oliguria. It is, therefore, a basic principle of calculus therapy to insist that the patient take increased amounts of fluid continually. The ability of the urine to dissolve sodium acid urate increases as the square of the volume. It is hard to control the excretion of the difficultly soluble salts. In the condition known as *oxaluria* the excretion of oxalate is marked and sporadic, often accompanied by nervous disturbances. Restriction of foods containing oxalates (Table 15) is of some assistance but may not be as effective as giving fluid, because considerable oxalate originates within the body.

The sedimentation or crystallization of salts from urine is not a true index of the tendency to calculus formation, since colloidal factors are involved and calculi are not made from sediments. Calculi and sediments

are separate phenomena equally influenced by the saturation conditions just discussed. Formation of urinary calculi involves crystallization following adsorption of the difficultly soluble constituents on a colloidal matrix or gel. The process commences around a central inciting nucleus which is often fibrin, sulfomucin, or bacterial protein. On rare occasions soft "protein stones" are found in human genito-urinary tracts; these represent the colloidal matrix not yet petrified by infiltration of difficultly soluble salts. Calculus formation is thus analogous to crystallization of salts in

TABLE 15

APPROXIMATE OXALIC ACID CONTENT OF FOODS

	MG. PER CENT
Spinach	900
Chard	650
Cocoa, rhubarb	500
Parsley	190
Beets	140
Figs	110
Chocolate, gooseberries	90
Okra, sweet potatoes	50
Carrots, celery, raspberries	35
Beans	30
Dandelion greens, endive, onions, oranges	25
Blackberries, currants, strawberries	20
Apricots, blueberries, grapes, plums	15

the protein matrix of bone; or, perhaps, it may more appropriately be likened to the pathological calcification of degenerating tissues or the deposition of insoluble gouty tophi in the gels of joint tissues. The most common urinary calculi contain large amounts of oxalate, phosphate, or urate. The stones usually show well developed Liesegang rings of precipitated porphyrin pigments or urochrome, which formerly were misinterpreted as layers of the calculus added at intervals.

Renal concretions are more frequent in males, and they may form anywhere in the genito-urinary tract. They are prone to appear in the kidney pelvis, or, as bladder concretions, during infectious ammoniacal decomposition of bladder urine. Urinary calculi frequently accompany hyperparathyroidism and other disorders (renal rickets, Cushing's syndrome, hyperthyroidism, etc.) in which considerable calcium and phosphate are excreted. They can also result from the crystallization of acetylsulfapyridine during excessive sulfapyridine therapy with inadequate fluid intake. Prior to calculus formation there is probably a flocculation of denatured protein at the surface of urinary epithelia which have been rendered abnormal by infection, inflammation, vitamin A or magnesium deficit, or by nervous processes. Hyalin casts and, possibly, cylin-

droids are similar flocculates from diseased kidney tubules. The presence of foreign surfaces such as fibrin, bacteria, rubber, or air can initiate flocculation of the small amounts of the colloidal sulfomucin which exists in normal urine in a poorly protected state. This effect is similar to the initiation of blood coagulation at foreign surfaces. The tendency of the insoluble inorganic suspensoids of urine to flocculate with sulfomucin and other proteins is offset by certain peptizing agents, especially by chondroitinsulfuric acid. It is claimed that the administration of large quantities of citrates inhibits calculus formation.

Ultzmann's method of burning the calculus is a rapid procedure for identifying the major components. A blue flame with sulfurous odor signifies cystine; a yellow flame with an odor of burnt hair indicates protein; while combustion with a colorless flame and no odor indicates uric acid. Stones that will not burn include carbonates which effervesce with hydrochloric acid, oxalates which effervesce when heated, and phosphates which do not effervesce. Actually, most urinary calculi are mixtures but a main component can usually be identified.

BILIARY CALCULI

The process of calculus formation in the gallbladder and biliary tracts is very similar to that described for urinary calculi, although the calculus constituents are different. Bile contains the excellent peptizing agents, protein, lecithin, and bile salts; but it is supersaturated with the very insoluble substances, cholesterol and calcium salts of the bile pigments. The frequent bacterial invasions of the biliary tract are prone to upset colloidal protection, causing the formation of flocculates and fibrin gels. Another important factor is the action of the gallbladder in concentrating its solids from two to ten times. The normal lyophilic protective substances of the bile are rendered ineffective by the introduction of ovalbumin, bacterial protein, or the serum albumin from inflammatory processes. These proteins initiate flocculation of the colloidal cholesterol and calcium bilirubinate.

Biliary calculi usually contain large amounts of crystallized free cholesterol and variable small quantities of calcium bilirubinate. Occasionally, almost pure bilirubinate stones are found. All types contain a protein gel matrix with protein and bilirubinate in the nucleus. Biliary calculi may occur anywhere in the biliary system, but they are most frequently found in the gallbladder.

Surgical removal of urinary and biliary calculi is not necessarily a permanent solution to the inciting pathology. It solves the acute crisis but the problem of diseased or abnormal tissue remains, and this requires dietary and hygienic considerations. Removal of the gallbladder is more successful because it remedies stasis and concentration of the bile. During

stasis, the bile salts are reabsorbed from the gallbladder into the blood stream. As the concentration of these peptizing agents decreases, the unprotected biliary cholesterol begins to crystallize.

COLLOID CHEMISTRY IN HISTOLOGY

The aim of histological technique is to render protoplasmic structures visible. To produce tissue pictures representative of living protoplasm requires consideration of colloidal reactions. The reversible sols and gels of living cells are sensitive systems which are easily flocculated or rendered abnormally permeable. Histological reagents are not unique in this respect; even the physiological entrance of water and salts into living cells affects their ultramicroscopic structure. The historic granular, alveolar, and fibrillar theories of protoplasmic structure were based on particular flocculation patterns of identical cell colloids. Fibrillar patterns are frequently the reaction products of reagents with adjacent sol droplets. Osmic acid has less tendency to flocculate protoplasm than most other fixing agents; at least, its flocculates remain in a submicroscopic state. However, the application of this fixing agent is limited by its feeble penetrating power. Osmic acid fixation, frozen sections, or gelatin imbedding are the preferred procedures for studying cell lipides.

The rates of diffusion of histological reagents are important considerations in staining. Reacting substances at times form flocculation membranes and the dyes diffuse toward these initial flocculation zones, thereby lowering their concentration at other points in the protoplasmic gel. The flocculation zones can be displaced by appropriate alterations in reagent concentration. The existence of supersaturated zones leads to rhythmic Liesegang flocculates, such as the Frommann lines in nerve fibers. Exogenous flocculates at the surfaces of tissue structures are also encountered. Both the particle size of the dye and the degree of swelling of the substrate are important in staining. Fat-soluble dyes stain lipid structures by dissolving in them, a portion of the lipid being simultaneously extracted by the staining solution.

The hydrogen ion concentration is a most important factor in successful histological technique. Fixing solutions more acid than pH 1.0 produce exaggerated membrane structures and sharply delineate phase boundaries; above pH 3.0, more homogeneous patterns are obtained. Histological substrates are classified as *acidophile*, *basophile*, or *neutrophile*, according to the electrostatic charges of the dyes which they select. The protein and phospholipide substrates in cells are ampholytes and they adsorb and flocculate either acid or basic dyes according to the pH of the staining reagent. The terms *acidophile* and *basophile* are, therefore, relative designations of the electrostatic condition of the histological substrates in the particular staining reagent used. Acid dyes stain best in acid solu-

tions, which render proteins electropositive; basic dyes react best in alkaline solutions, where the proteins are electronegative.¹ The isoelectric points of many tissue constituents fall in the dilute acid range (Table 10, page 47). These substances are electronegative and readily adsorb basic dyes, except in sufficiently concentrated acid solutions. Most tissue constituents are stained diffusely by acid dyes. The polysaccharide-sulfuric acid components of the sulfomucins and of the interfibrillar substance of hyaline cartilage, and the nucleic acid constituents of chromatin render these structures basophilic. Alcohol fixation alters the isoelectric points of certain tissue constituents, perhaps by removing lipid films. Formaldehyde destroys the basic amino radicals of proteins and thus renders the tissue structures more basophilic. Mercuric chloride and picric and chromic acids have an opposite effect. *Mordants* are metallic salts or hydroxides which form complexes with amphoteric dyes and accentuate their latent basic properties. This permits the staining of basophilic structures by modified acid dyes.

Silver impregnation methods are frequently employed in histology. Small nuclei of insoluble silver salts are first formed by *argentophile* proteins, chlorides, phosphates, and the like, and these are reduced to metallic silver by the action of light or of reducing agents. The small nuclei grow, as in photographic development, and additional silver is deposited about them. The distribution of argentophile substances in cells can be changed by fixing agents which dissolve cell lipides or remove protective colloids and thus open new diffusion paths for the silver reagent. With increasing particle size, the color of the precipitated silver changes from yellow to black.

The histologist has at his command certain procedures, such as tissue culture and vital and supravital staining, to confirm and correct the fixed tissue pictures. *Vital staining* is of particular physiological interest. The distribution of a vital dye in cells is partly determined by solubility and adsorption. Penetration of dyes into living cells is subject to embolic effects. Basic vital dyes (Janus green, methylene blue, and neutral red) are useful for delineating preformed tissue structures, at whose surfaces they flocculate. Acid vital dyes (carmin, trypan blue, water blue, and Sudan III) aid in the study of transport problems. They tend to stain cells diffusely and, at high concentrations, they flocculate as vacuolar structures. They also specifically stain certain exceptionally amphoteric cells, as, for example, the cells of proximal convoluted renal tubules, histiocytes, and reticulo-endothelium. Dyes which do not permeate living

¹ *Acid dyes* include acid fuchsin, alizarin, alizarin red, water-soluble aniline blue, azocarmine, carmin, Congo red, eosin, erythrosin, lichtgrün, Lyon's blue, methyl blue, orange G, phloxine, picric acid, pyrrol blue, trypan blue, and water blue.

Basic dyes include Azur I and II, basic fuchsin, Bismarck brown, brilliant cresyl blue, cresyl echt violet, gentian blue, gentian violet, Janus green, methylene blue, methyl green, methyl violet, neutral red, Nile blue, pyronin, safranin, thionin, and toluidine blue.

tissues are flocculated extracellularly and are phagocytized by histiocytes. Attempts to determine oxidizing and reducing localities in cells by methylene blue, permanganates, and so forth, are complicated by problems of redistribution and side reactions.

Histological technique is a special application of colloid chemistry. Like most biological sciences, it began with empiricism; but today it employs carefully standardized reagents and makes use of frequent comparisons of fixed and stained designs with unaltered cells, frozen specimens, and spodograms.

ENZYME CHEMISTRY

"Vitalism can no more increase the corpus of our knowledge than imaginary delicacies can provide sustaining food for our bodies." — MORRIS R. COHEN

CATALYSIS

Catalysts are substances which alter the velocity of chemical reactions. In ordinary usage, the term refers to positive catalysts which accelerate reactions. An ideal catalyst, while participating in the reaction, is neither destroyed nor permanently altered. Actually, catalysts are gradually consumed by secondary reactions and seldom do they catalyze more than 200,000 times their own weight of reacting material. For this reason, they must eventually be replaced or regenerated. No energy is derived from the catalyst, hence it does not alter the equilibrium point of a reversible reaction. Catalysts form intermediate, highly reactive compounds; and, when present in colloidal form, they initiate adsorption reactions.

The importance of the surface area of colloidal catalysts is indicated by the following illustrations: The heat of adsorption of oxygen on charcoal is intermediate between the heat of combustion of solid carbon and that of gaseous carbon, showing that, at the surface of the charcoal, certain carbon atoms approach the reactivity of the gaseous state. Smooth sheets of platinum do not cause liberation of oxygen from hydrogen peroxide, whereas colloidal platinum decomposes it violently. In colloidal catalytic systems, the materials which are to react are first adsorbed on the colloidal particles; hence, adsorption and diffusion become limiting factors in catalysis.

ENZYMES

In vitro hydrolyses of the high molecular proteins, polysaccharides and complex lipides require vigorous chemical treatment. This is incompatible with living protoplasm, yet the latter rapidly accomplishes such reactions by means of its catalytic enzymes. The early experimental work on enzymes centered about studies of sugar fermentation, the discovery of micro-organisms, and the subsequent separation of enzymes as non-

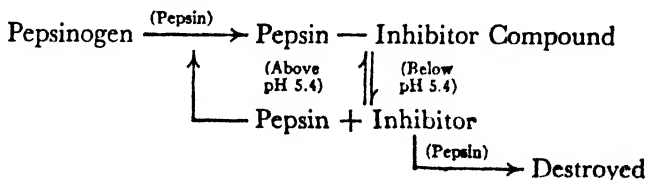
living catalysts. Research was stimulated by the discovery that antiseptics have a greater inhibiting effect on bacterial life than on individual enzymes; and the crystallization of a number of pure enzymes has completed the transition from empirical biology to scientific enzyme chemistry.

Enzymes are defined as catalysts produced by living cells; they initiate and organize the complex chemical changes in cells which we term *metabolism*. There are two general phases of metabolism: *anabolism*, or synthetic chemical processes which construct complex molecules from simple units; and *catabolism*, or hydrolytic and oxidative processes which fragment molecules. An enzyme differs from an ordinary laboratory catalyst in that the former acts specifically on a certain substance which is termed its *substrate*. Enzymes can select their particular substrates from a mixture of stereoisomers. Enzymes are divided into two biological classes: *extracellular* enzymes, secreted by cells; and *endocellular* or *intracellular* enzymes, which normally are retained within the cells.

Zymogens and Their Activation

Enzymes are found frequently in living cells as inactive precursors, the *zymogens*. These are activated either by colloidal substances, the *kinases*, or by dialyzable, heat-stable substances, the *coenzymes*. We have previously encountered a kinase effect in the activation of prothrombin by thrombokinase; another example is the activation of trypsinogen by enterokinase to form active trypsin. Enterokinase exists in pancreatic juice as an inactive precursor, prokinase, which is activated in the intestinal mucosa to form the active kinase.

The conversion of trypsinogen to trypsin is an autocatalytic reaction; that is, trypsin itself can activate trypsinogen and the chymotrypsinogens in the absence of inhibiting impurities. Effective conversion of trypsinogen by trypsin requires the presence of calcium ions. In mixtures such as pancreatic juice, enterokinase becomes the chief accelerator. Enterokinase has been shown to be an enzyme which hydrolyzes trypsinogen to form two proteins, the active trypsin and an inactive protein fragment. Trypsin performs the same reaction, autocatalytically. The transformation of chymotrypsinogen to chymotrypsin involves the splitting of peptide bonds and the freeing of five amino radicals. The conversion of pepsinogen to pepsin, in the presence of sufficient hydrogen ions, is also autocatalytic. Here the reaction product, pepsin, is the true accelerator; the reaction is as follows:



There is also a similar trypsin inhibitor which is a polypeptide; an anti-trypsin or inhibitory polypeptide is present in certain intestinal parasites. (See page 89.)

Coenzymes operate by taking part in the chemical reaction catalyzed by the enzyme; some are specific, but many are non-specific. Of those listed in Tables 17 and 20, page 93, the bile salts, adenylic acid, and cocarboxylase are rather specific coenzymes for lipases, phosphorylases, and carboxylase, respectively. The inorganic ions, iron-porphyrin complexes, cozymases, sulfhydryl ($-SH$) compounds, and flavoproteins may each activate a number of different enzymes.

Many enzymes are definite chemical compounds of protein and a coenzymic radical; the latter is called the *prosthetic radical*. Thus catalase, peroxidases, and cytochrome oxidase contain prosthetic iron-porphyrin complexes; and the yellow enzymes have flavin nucleotide radicals. The iron-porphyrin-protein complexes include some of the most active enzymes. Thus, catalase and cytochrome oxidase have very high turnover numbers (molecules of substrate transformed per mol of enzyme per minute, Table 16).

TABLE 16

APPROXIMATE TURNOVER NUMBERS OF ENZYMES

ENZYME	MOLECULES SUBSTRATE TRANSFORMED PER MINUTE
Catalase	2,500,000
Cytochrome oxidase	2,300,000
Peroxidase	150,000
Polyphenol oxidase	3,000 to 70,000
Ascorbic oxidase	50,000
Phosphorylase	40,000
Tyrosinase	40,000
Hexosemonophosphate dehydrogenase	29,000
Laccase	25,000
Alcohol dehydrogenase	20,000
Triosephosphate dehydrogenase	20,000
Enolase	10,000
Myokinase	10,000
Aldolase	8,800
<i>d</i> -Amino acid oxidase	2,000
Cytochrome <i>c</i>	1,400

Some ions inhibit enzyme action. For example, the cations of mercury, silver, and gold; the cyanide, fluoride, and iodoacetate anions; and protein precipitants, such as picric and phosphotungstic acids, are all enzyme inhibitors. The effects of ions are often, but not always, reversible. Fluoride is regarded as an inhibitor of phosphatases; phlorhizin of phosphorylases; iodoacetate of phosphorylases and dehydrogenases; azide,

TABLE 17
HYDROLYTIC ENZYMES

ENZYMES	SUBSTRATE	REACTION PRODUCTS	ACTIVATORS
<i>Esterases</i>			
Adenosine triphosphatase (myosin)	<i>Ester linkages</i> Adenosine triphosphate	Adenosine diphosphate, phosphoric acid	Ca ⁺⁺
Cholesterase	Sterol esters	Sterol, acid	Ca ⁺⁺ , Mg ⁺⁺ , Mn ⁺⁺ Bile salts
Choline esterase	Choline esters	Choline, acid	
Lipases (steapsin, etc.)	Fats	Glycerol, fatty acids	
Lecithinase A	Phospholipides	Lysophospholipide, fatty acid	
Lecithinase B	Phospholipides	Glycerophosphorylcholine, fatty acids	
Nuclease (polynucleotidase)	Nucleic acids	Nucleotides	Mg ⁺⁺ , Mn ⁺⁺
Phosphatases	Phosphoric esters	(Sugar, etc.), phosphoric acid	
5-Nucleotidase	5-Phosphoribosides	Nucleosides, phosphoric acid	Mg ⁺⁺
Pyrophosphatases	Pyrophosphate esters	Phosphate esters, phosphoric acid	Mg ⁺⁺ , adenylic acid
Phosphorylases	Sugar, phosphoric acid	Phosphate, esters, water	
Sulfatases	Sulfuric esters	(Phenol, etc.), sulfuric acid	
Chondroitin sulfate	Sugar sulfates	Sugar, sulfuric acid	
<i>Carbohydrases</i>			
Glycosidases	<i>Glycoside linkages</i> Simpler glycosides	Galactose (sugar or aglycone) Glucose (sugar or aglycone)	CNS-
α -Galactosidase	α -Galactosides	Monosaccharide (sugar or aglycone)	
α -Glucosidase (maltase)	α -Glucosides	Monosaccharide (sugar or aglycone)	
β -Glycosidase (emulsin, lactase)	<i>trans</i> -Glycosides	Monosaccharide (sugar or aglycone)	
Glycuronidases	Glycuronides	Uronic acids (sugar or aglycone)	
α -Mannosidase	α -Mannosides, 2-deoxyglycosides	Monosaccharide (sugar or aglycone)	
Nucleosidases	Nucleosides	Monosaccharide (purine or pyrimidine)	
Sucrase (invertase)	β -Fructofuranosides	Fructose, other sugar	
<i>Polysaccharases</i>			
Amylases (ptyalin, amylopsin)	<i>Polysaccharides</i> Dextrin, glycogen, starch	Maltose	Cl ⁻ , unknown coenzyme
Cellulase	Cellulose	Cellobiose	
Chitinase	Chitin	Chitobiose, glucosamine	

Cytases	Hemicelluloses	Monosaccharides	
Inulase	Inulin	Fructose	
Mannase	Mannans	Mannose	
Pectase	Pectins	Pectic acid, monosaccharides	
Specific polyases	Prosthetic polysaccharides	Aldobionic acid, monosaccharides	
Xylanase	Xylans	Xylose	
Proteases	<i>Peptid linkages</i>		
<i>Peptidases (exopeptidases)</i>	<i>Peptides</i>		
Aminopeptidase ¹	Peptides	Amino acids	
Carboxypeptidase ²	Peptides	Amino acids	
Protaminase	Protamines	Peptides	
Dipeptidase	Dipeptides	Amino acids	Mn ⁺⁺
Leucyl peptidase	Leucyl dipeptides	Amino acids	Mn ⁺⁺ , Mg ⁺⁺
Prolidase ³	Prolyl peptides	Amino acids	Mn ⁺⁺
Prolinase ⁴	Prolyl peptides	Amino acids	
<i>Proteinases (endopeptidases)</i>	<i>Proteins</i>		
Cathepsins	Isoelectric protein	Proteoses, peptones	—SH, vitamin C
Papainases	Proteins	Proteoses, peptones	—SH, CN ⁻
Pepsinases (pepsins)	Protein cations	Proteoses, peptones	H ⁺ , pepsin
Specific proteinases	Specific proteins		
Antithrombin	Thrombin		
Enterokinase	Trypsinogens	Trypsin, protein	
Renin	Hypertensinogen	Hypertensin (angiotenin)	
Renin	Casein	Paracasein	
Thrombin	Fibrinogen	Fibrin	
Trypsinases (trypsin)	Protein anions	Polypeptides	
<i>Amidases (hydrolytic deaminases)</i>	<i>Special N—C linkages</i>		
Arginase	Arginine	Ornithine, urea	Ca ⁺⁺ , thrombokinas
Asparaginase	Asparagine	Aspartic acid, ammonia	Enterokinase, trypsin, OH ⁻
Glutaminase	Glutamine	Glutamic acid, ammonia	
Glycocyaninase	Glycocyamine	Glycine, urea	
Hippuricase (histozyme) ⁵	Hippuric acid	Glycine, benzoic acid	
Urease	Urea	Carbon dioxide, ammonia	—SH, Mn ⁺⁺

¹ Hydrolyzes peptides at amino end of chain.² Hydrolyzes peptides at carboxyl end of chain.³ Requires NH = radical of proline in peptide linkage.⁴ Requires carboxyl radical of proline in peptide linkage.⁵ While hippuricase acts upon a peptide linkage, it is usually classified as an amidase.

carbon monoxide, cyanide, and sulfide of enzymes containing copper or iron; *p*-aminobenzoate, diethyldithiocarbamate, ethylxanthate, and sulfonamides of enzymes containing copper; and *p*-aminophenol of flavo-protein enzymes. However, many inhibiting substances are not entirely selective or specific in their action. Antiseptics to be used in enzyme experiments must be chosen with care. Chloroform inactivates certain enzymes; toluene is least harmful to a variety of enzymes and is, therefore, most generally used.

The protein enzymes contain labile groups, such as the —SH radical of cysteine, which can easily be converted to an oxidized form. The reduced form is specifically necessary for the activity of some enzymes, whereas the oxidized form is required for others; and the pH of the medium can affect the enzyme activity by altering the proportions of the two forms. The —SH radicals of enzymes are also inhibited by organic arsenical compounds and by iodoacetate and its derivatives. The activation of urease and other enzymes by —SH compounds or by cyanide anions is probably due to the formation of active reduced forms. The inactivation of enzymes by such oxidizing agents as iodine, hydrogen peroxide, and permanganates is a similar reversible effect. Some enzymes are inhibited by formaldehyde and nitrous acid, which destroy the free amino-radicals of proteins. Ultraviolet light and roentgen or radium rays inactivate enzymes by producing obscure chemical alterations.

Classification

Enzymes are frequently named from the substrates whose reactions they catalyze. They are at present divided into two general groups: the *hydrolytic enzymes*, which catalyze hydrolytic reactions; and the *oxidizing-reducing enzymes*, which catalyze the oxidation or reduction of their substrates. Enzymes often act in series to catalyze a system of coupled reactions; this is especially true of the oxidation-reduction enzymes to be discussed in the next section. The action of enzymes is usually limited to certain chemical radicals contained within their substrates, as indicated by the classification of hydrolytic enzymes in Table 17.

Crystalline Enzymes

To date, the enzymes alcohol dehydrogenase, aldolase, amylase, carbonic anhydrase, carboxylase, catalase, enolase, ficin, lactic dehydrogenase, lactoperoxidase, lysozyme, muscle phosphorylase, papain, peroxidase II, rennin, ribonuclease, tyrosinase, urease, yellow enzymes, carboxypeptidase, α -, β -, and γ -chymotrypsins, pepsins, and trypsin have all been crystallized, as have also the zymogens of pepsin, trypsin, and the chymotrypsins. Isolation has proved that enzymes are proteins; some are active in solutions so dilute that protein reactions are negative. Closely

related proteins can crystallize as solid solutions of mixtures which are difficult to separate, but such mixtures are usually detectable by solubility differences and some of the crystalline enzymes have been recrystallized many times without demonstrable change in their properties. A very few enzymes (taka-diestase and emulsin) are not destroyed by proteolytic enzymes and may, therefore, be non-protein in nature. A partial protein classification of enzymes is given in Table 18.

TABLE 18

PROTEIN CLASSIFICATION OF ENZYMES

PROTEIN	ENZYME
Albumins	Antithrombin, ribonuclease
Cupreins	Polyphenol oxidases, ascorbic oxidase, tyrosinase
Flavoproteins (yellow enzymes)	<i>d</i> -Amino acid oxidase, <i>l</i> -amino acid oxidase, cytochrome reductase, diaphorase, glucose oxidase, glycine oxidase, xanthine oxidase
Globulins :	Alkaline phosphatase, adenosine triphosphatase, pepsin, phosphorylase, thrombin, trypsin, urease
Hemochromogens	Catalase, peroxidases
Lipoprotein	Thrombokinas
Mucoid	Thrombin
Nucleoprotein (pyridine nucleotide proteins)	Dehydrogenases for carbohydrates and related substances
Porphyrin chromoprotein	Cytochrome oxidase
Prolamine	Papain
Proteose	Rennin

Adsorption

The protein enzymes are colloids whose preliminary action is the formation of adsorption compounds with their substrates. For example, when pepsin sol is added to a suspension of fibrin in dilute hydrochloric acid and the mixture is shaken vigorously and filtered, the pepsin is quantitatively removed from the solution by adsorption on the fibrin. If this adsorption complex is suspended in dilute hydrochloric acid, the second or chemical stage of the enzyme action follows. In the chemical reaction, the pepsin disintegrates the fibrin substrate into soluble digestion products. Enzyme activity is influenced by factors which affect adsorption, including changes in the concentrations of enzyme, substrate, and competing substances. Two very important factors are temperature and pH. These will be considered in detail.

Temperature

Usually, enzymes are most effective at temperatures between 38° and 45° C., although the optima for certain plant enzymes are much higher. The optimum temperature is the result of two different effects. It is well known that many chemical reactions are approximately doubled by a rise in temperature of 10° C. However, enzymes are heat-labile proteins which undergo chemical denaturation and oxidation at higher temperatures. The *optimum temperature* of an enzyme is the point at which increased reactivity is balanced by increased destruction of the enzyme. Most enzyme solutions are quickly denatured or otherwise inactivated when they are kept at 70° C.; and their complete destruction by boiling serves as a general test for the presence of enzymes. Impurities in enzyme solutions often render heat denaturation irreversible. Crystalline trypsin is inactivated when heated briefly at 70° to 100° C. but regains its activity on cooling. Dry enzyme preparations can withstand high temperatures, and even in water enzymes are not destroyed by lowering the temperatures to - 190° C.

pH

Since enzymes are amphoteric colloids, they are sensitive to pH changes. The pH at which an enzyme is most effective is termed its *optimum pH*. (See Table 19 for compilation.) There is often a rough correspondence between the optimum pH and the pH of the natural medium in which the enzyme is found. For example, the pH of the gastric juice of adults corresponds to the optimum pH for pepsin. Pepsin and rennin, having relatively low pH optima, attack the protein cations which preponderate at the pH of gastric juice; whereas trypsin attacks protein anions which are formed at the pH of the intestinal contents. (Compare values in Table 6, page 25, and Table 19, page 87.) It is obvious that the activity of proteolytic enzymes is at least partly dependent on the isoelectric points of the particular protein substrates undergoing digestion. Further relations of pH to digestion will be considered in the next chapter.

Reversibility

It has been demonstrated that certain hydrolytic enzymes can act reversibly under appropriate conditions. An enzyme may cause hydrolysis or synthesis depending on the pH of the mixture and the concentrations of substrate, hydrolytic products, and enzyme. Examples of partial resynthesis of the hydrolytic products of enzyme action *in vitro* are: maltose isomers from glucose, by maltase; synthetic peptones (called plasteins) from amino acids, by trypsin and pepsin at pH 4.5; β -methylglucoside from glucose and methyl alcohol, by emulsin; neutral fat from fatty acids and glycerol, by lipase; and glycerophosphoric acid from glycerol and

phosphoric acid by phosphatases. These syntheses are relatively slow and require large concentrations of the reverse substrates.

TABLE 19

OPTIMUM pH OF ENZYMES

Adenylase	6.0	Histidase	8.5
Aldehydemutase	6.5	Histidine decarboxylase	8.8
<i>d</i> -Amino acid oxidase	9.0	Histozyne	7.0
Aminopeptidase (intestine)	8.0	Hyaluronidase	5.8
Amylase (blood, muscle)	7.0	Hypertensinase	8.0
Amylase (liver)	6.0	Inulase	4.0
Amylase (malt)	4.5	Invertase (intestine)	6.8
Amylopsin (pancreatic amylase)	7.0	Invertase (yeast)	4.5
Arginase	9.8	Kynureninase	7.3
Arginine decarboxylase	5.3	Laccase	7.5
Ascorbic oxidase	5.5	Lactase (intestine)	5.5
Asparaginase	8.0	Lactic dehydrogenase	8.0
Aspartase	7.0	Lipase (gastric)	5.0
Autolytic enzymes	4.5	Lipase (ricinus)	5.0
Bromelin	6.0	Malic dehydrogenase	6.6
Carbonic anhydrase	8.1	Maltase (intestine)	6.0
Carboxylase	6.1	Maltase (yeast)	6.5
Carboxypeptidase (pancreas)	7.4	Myosin	9.0
Carotene oxidase	6.5	Nucleosidase, purine (pancreas)	7.5
Catalase	7.0	Nucleosidases (other tissues)	6.5
Cathepsin (leukocyte)	3.0	Ornithine decarboxylase	5.3
Catheptic pepsinase (kidney, spleen)	4.0	Papain	5.5
Catheptic trypsinase (kidney, spleen)	4.9	Pepsin	2.0
Cellulase	4.5	Peroxidase (vegetable)	5.0
Chitinase	5.2	Phosphatase (acid)	5.0-6.0
Choline esterase	8.4	Phosphatase (alkaline)	8.5-9.5
Citric dehydrogenase	8.5	Phosphatase (erythrocyte)	6.5
Desulfurase	7.2	Prolinase	7.8
Dipeptidase (intestine)	7.5-8.0	Ptyalin	6.6
Emulsin	4.4	Pyrophosphatase	8.0
Esterase (liver, pneumococcus)	7.5	Rennin	3.5
Esterase (muscle)	6.0	Ribonuclease	7.5
β -Glucosidase	5.0	Steapsin (pancreatic lipase)	8.0
Glutamic decarboxylase	4.3	Succinic dehydrogenase	7.5
Glutaminase	7.5	Transaminase (aminopherase)	7.5
Glyoxalase	8.0	Trypsin	8.0
Guanase	9.2	Tryptophanepyrrolase	6.8
Guanylase	5.3	Tyrosinase	7.0
Histaminase (diamine oxidase)	7.1	Urease	7.0
		Uricase (two optima)	8.9 and 10.0
		Xanthine oxidase	7.0

Determination

Under controlled conditions the rate of an enzymic reaction is proportional to the enzyme concentration. Large concentrations of the reaction products noticeably retard the enzyme activity because they are partially adsorbed on the enzyme particles and render the latter inactive. Enzyme concentrations are determined and expressed in relation to arbitrary standards of activity, using the time required to produce a definite degree of conversion of an appropriate substrate or the quantity of the preparation necessary to produce such conversion in a specified time. Detailed methods of determining enzymes are given in the references.

The clinician is occasionally interested in determining the concentrations of the following enzymes: pepsin and rennin in gastric contents; amyllopsin, steapsin, and trypsin in intestinal contents; amylase, esterase, lipase, and phosphatase in blood; and amylase in urine. Such enzyme determinations have been employed in studies of gastro-intestinal, hepatic, pancreatic, prostatic, and bone diseases. Serum lipase and esterase are low in liver diseases, vitamin A deficiency, malignancy, and in diabetes mellitus, and high in acute pancreatitis and pancreatic carcinoma; serum amylase is low in hepatic disease, and increased by high intestinal obstruction, parotitis, renal damage, or certain pulmonic conditions, also blood and urine amylase are high in acute pancreatitis and in diabetes; blood alkaline phosphatase is increased in rachitis, hyperthyroidism, Paget's disease, osteoblastic bone sarcoma, and obstructive jaundice; and serum acid phosphatase is elevated in prostatic malignancy. Steapsin and erythrocytic lipase are not inhibited by atoxyl, whereas blood esterase and hepatic lipase are inhibited by this substance and by quinine and strychnine.

ENZYME FUNCTION

"The facts of nature will always burst the narrow bonds of human theories." — SIR JAMES G. FRAZER

GENERAL ENZYME FUNCTIONS

A discussion of the functions and pathology of enzyme activity would involve most of the subject matter of medicine; specific details are, therefore, considered in the various metabolic sections.

Enzymes initiate and partly control almost every conceivable physiological process. Digestion in the gastro-intestinal tract is a series of enzymatic hydrolytic processes. Oxidations, syntheses, transformations, and fragmentations of foods by tissues are often complex or coupled reactions in which the activities of a variety of enzymes are co-ordinated. The —SH coenzymes for proteolytic cathepsins inhibit certain oxidizing enzymes (Table 20, page 93). The reduced forms of these coenzymes increase

in amount during the autolytic digestion of asphyxiated cells, and they assist in accelerating the proteinase activity. Autolysis of tissues will be considered more fully on page 421.

Synthetic processes in cells are incompletely understood. Cathepsins are presumed to be the enzymes which synthesize proteins when suitable energy sources are available. That protein synthesis is directed by the cell proteins (templates) is deducible from the fact that very specific proteins are produced during growth, reproduction of gene materials (the enzyme carriers of heredity), and in the duplication of nucleoprotein viruses. The mammary gland contains little lipase or lactase, yet it produces considerable amounts of milk fat and of lactose. It has been found that this gland does not make its lactose from glucose and galactose by reversing a lactose-hydrolyzing mechanism, but rather, from lactic acid by synthetic processes. Similarly, the common metabolic phosphorylations are attributable to phosphorylases, rather than to reverse activity of phosphatases.

Since most enzymes are proteins, their parenteral injection stimulates immune mechanisms to produce *antienzymes*. Careful immune studies with crystalline urease demonstrate the production of a specific antiurease, which inhibits the activity of the enzyme. Crystalline ribonuclease is also inhibited by its antienzyme, but the antibodies for crystalline catalase and lung thrombokinasase do not impair the action of these enzymes. There is some evidence that so-called antitryptic substances are not always true immune bodies (page 81). Antitrypsin has been found in blood, intestinal mucosa, parasites, and egg white. In egg white, antitrypsin has the interesting role of regulating the digestion of ovalbumin to provide a gradual release of the precious store of protein-bound water and thus to maintain proper economy in the growing embryo.

Viruses, bacteriophages, and the nucleoproteins concerned in heredity are specific proteins of enzymatic nature. Owing to the special terminology and the knowledge of protein structure required, the discussion of these substances is given on pages 492 to 498. Modern genetic theories postulate genes to be the activities of enzymatic molecules which exist in limited numbers in any given cell (sometimes as few as one or two specific giant colloidal molecules). Calculations of the sucrase content of certain cells show that relatively few molecules of some ordinary enzymes are to be expected in living cells. The distribution of enzymes in the nucleus of rat liver cells has been studied by modern methods. The nuclei contain arginase, acid and alkaline phosphatases, cytochrome oxidase, esterase and lactic acid dehydrogenase, but no catalase or succinic acid dehydrogenase.

Enzyme Adaptation

Interesting studies of adaptations in enzyme production have been made with micro-organisms. Slow enzymic adaptation is traceable to

natural selection of a low proportion of mutants in cultures. Hence, growth in an appropriate medium can give rise to *mutabile* strains of micro-organisms after a lag period. Yeast grown in the presence of small quantities of cyanide develops into a new substrain without cytochrome activity.

There is also a more rapid and direct adaptation of enzyme production related to the chemical environment. Thus, growth of glucose-utilizing organisms on sugars such as galactose or xylose stimulates the formation of specific *adaptive enzymes*, which allow the fermentation of these sugars. During the process, the normal or *constitutive enzyme* for the utilization of glucose is not lost. Chemical adaptation of this type does not require the selective growth of a mutant. It may be compared to the well-known diminution in anaerobic utilization of glucose upon exposure of a cell to oxygen. This *Pasteur effect* (page 315) can be reversed in certain micro-organisms by means of cysteine or other reducing agent, which converts the enzyme concerned into its active reduced form. Formation of enzymes by chemical adaptation is possibly due to mass action effects, provided the enzyme is formed from a precursor and is present only in traces before the adaptation. Introduction of the substrate or the end products of the reaction, which can unite specifically with the enzyme, should disturb the equilibrium and increase the rate of formation of the enzyme. Either starch or maltose increases amylase production by *Aspergillus niger*, and either sucrose or fructose can accelerate invertase production by yeast. Chemical adaptation by micro-organisms does not occur in the presence of antiseptics. Applications of adaptive enzymes are mentioned on pages 441, 471, 474 and 478.

CHEMISTRY OF OXIDATION

"As nature is not primarily concerned with answering our questions, ideal crucial experiments are rare. The discovery of what has hitherto been unknown involves a leap into the dark, and the tragic history of human failure to solve our vital problems shows how real the darkness is." — MORRIS R. COHEN

INTRODUCTION

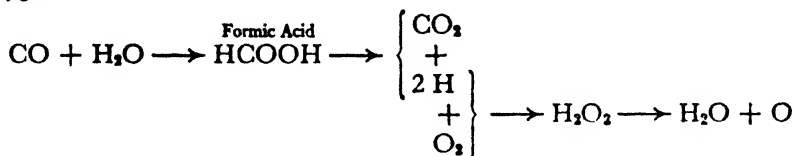
Life is dependent on energy exchanges whose chief ultimate manifestations are heat and mechanical work. However, some energy is always used for surface, osmotic, chemical, and synthetic activities, and for nervous and glandular action. The sources of energy are chemical reactions within the living cells, principally the stepwise oxidation of foods. The oxygen used in the aerobic reactions of cells is, therefore, a gaseous food. The nitrogen of the inspired air is of value only as an inert diluting gas except in a few micro-organisms. Gaseous oxygen is often poisonous to anaerobic cells, which utilize the chemically bound oxygen of foods; aerobic cells are sensitive to anoxic conditions. Certain cells of the respiratory center in the medulla are permanently injured by short periods of

serious oxygen deficit, as in drowning. Hydrolytic reactions catalyzed by the enzymes discussed in the preceding section usually cause only small energy changes. A few exceptional hydrolyses, such as the splitting of phosphocreatine and adenosine triphosphate, have appreciable heats of reaction (page 318).

The animal is a complicated chemical engine which transforms energy into chemical and mechanical forms and ultimately into heat, in accordance with the law of the conservation of energy. For this reason, the biochemical sciences of nutrition and dietetics evaluate foods in terms of calories. Before discussing energy metabolism, the oxidative mechanisms responsible for the internal respiration of cells will be considered. The preliminary external respiration in the lungs and the transport of carbon dioxide and oxygen by the circulatory system are discussed on pages 23 and 543, respectively.

OXIDATION-REDUCTION REACTIONS

Oxidation of a molecule involves either combination with oxygen, or a loss of hydrogen or of electrons. During oxidation by any of these processes the molecule reduces another substance, that is, hydrogen or electrons are transferred from the substance being oxidized to the substance which is reduced. Thus, when carbon monoxide is burned in the presence of palladium as catalyst, the carbon monoxide is oxidized and oxygen is reduced:



In such a reaction, the oxidized and reduced substances are termed *reductant* and *oxidant*, or *oxygen acceptor* and *hydrogen acceptor*, respectively.

Often the oxidation of organic materials is not a single stage chemical process, but a series of separable linked reactions many of which are reversible. Each step is a source of energy and represents an essential catalytic link in the oxidation. A mixture of the oxidized and reduced forms of any intermediate in the oxidation chain is an *oxidation-reduction system*. Each oxidation-reduction system has a characteristic chemical potential, that is, avidity for electrons. The *oxidation-reduction potential* of the system is a quantitative estimate of its oxidizing or reducing power. Systems with high reduction potentials reduce systems which have lower potentials. The potential difference between two such systems can be determined, and expressed as voltage, by reference to the standard hydrogen electrode. Detailed discussions may be found among the references.

Certain dyes can be used as *oxidation-reduction indicators* because the

oxidized and reduced forms have different colors. Indophenols, methylene blue, indigosulfonates, nitro compounds, and safranines are useful indicators for studies of the oxidation processes of living materials. The reactants of biological systems are often ions whose concentrations and ratios of oxidized to reduced forms are affected by the pH of the medium. The hydrogen ion concentration may have, therefore, a marked influence on oxidation-reduction systems, and also on the oxidation-reduction indicators.

In living cells the reversible oxidizing-reducing systems act as *carriers*, *mediators*, or *bridges* by which hydrogen and electrons are transferred. This makes possible the oxidation of foods by the relatively inactive molecular oxygen transported to the tissues. In general, it is only the electrons of the substrate which are finally transferred to oxygen; the hydrogen of the foods can enter or leave the acid-base continuum as protons at various points in the oxidative chain. The hydrogen of the end product (water) has two immediate sources, namely, from protons of the aqueous continuum and from hydrogen peroxide through the action of catalase.

DEHYDROGENASES

Outside the cells, foodstuffs do not readily enter into energy-yielding reactions with molecular oxygen, from which it appears that special cellular provisions are necessary. These are the oxidizing-reducing enzyme systems of Table 20. It was stated above that in carbon monoxide oxidation the intermediate substance, formic acid, is oxidized by liberation of hydrogen. There is a similar activation and removal of hydrogen from foods in living cells. The transformation of ordinarily inert food into active reducing forms explains how certain anaerobic biological oxidations continue in the complete absence of oxygen. The typical *anaerobic dehydrogenases* are enzymes which activate food hydrogen in the presence of oxidation-reduction systems (cozymase, cocarboxylase, etc.) capable of accepting the hydrogen and accelerating the reaction. Examples of this important group of enzymes are given in Table 20. Dehydrogenases are specific enzymes whose activities are reversibly inhibited by narcotics, and by substances chemically related to their specific substrates. Morphine inhibits the activity of citric, glucose, and lactic dehydrogenases. Arsenite inhibits amino acid dehydrogenases; sufficient iodoacetate inactivates numerous carbohydrate dehydrogenases, and several are specifically susceptible to dilute iodoacetate (Table 20).

Dehydrogenases can be detected in tissue preparations, in evacuated tubes, by the addition of an oxidation-reduction indicator, such as methylene blue, to the anaerobic mixture. The food hydrogen is activated and transferred to the methylene blue, which is reduced to a colorless or leuko form. When molecular oxygen is subsequently admitted to the mixture, the leuko methylene blue is rapidly reoxidized to the colored

TABLE 20

OXIDIZING AND REDUCING ENZYMES

	SUBSTRATE	REACTION PRODUCTS ¹	HYDROGEN CARRIER
<i>Anaerobic dehydrogenases</i>			
Alcohol dehydrogenase ²	Alcohol	Acetaldehyde, H	Cozymase I
Citric dehydrogenase ³	Citric acid	α -Ketoglutaric acid, CO ₂ , H	Cozymase I
Fatty acid dehydrogenase	Fatty acids	Unsaturated fatty acids	
Formic dehydrogenase	Formic acid	CO ₂ , H	Cozymase I
Formic hydrocouplase	Formic acid	CO ₂ , H ₂ (gas)	Cozymase I
Glucose dehydrogenase ⁴	Glucose	Gluconic acid, H	Cozymase I or II
Glutamic dehydrogenase	Glutamic acid	α -Ketoglutaric acid, NH ₃	Cozymase I or II
Glycero-phosphate dehydrogenase	α -Glycerophosphoric acid	Phosphoglyceraldehyde, H	Cozymase I
Hexosephosphate dehydrogenase	Hexosemonophosphoric acids	Phosphogluconic acid, H	Cozymase II
Hydrogenase	H ₂ (gas)	H	
β Hydroxybutyric dehydrogenase	β Hydroxybutyric acid	Acetoacetic acid, H	Cozymase I
Lactic dehydrogenase ⁵	Lactic acid	Pyruvic acid, H	Cozymase I
Malic dehydrogenase	Malic acid	Oxal-acetic acid, H	Cozymase I
Phosphogluconic dehydrogenase	Phosphogluconic acid	Ketophosphogluconic acid, H	Cozymase II
Purine deaminases ⁶			
Adenase	Adenine	Hypoxanthine, NH ₃	
Adenosine deaminase	Adenosine	Inosine, NH ₃	
Adenylases	Adenylic acids	Inosinic acids, NH ₃	
Guanase	Guanine	Xanthine, NH ₃	
Guanosine deaminase	Guanosine	Xanthosine, NH ₃	
Pyruvic dehydrogenase	Pyruvic acid	Acetic acid, CO ₂ , H	Cornacoxylase, Mg
Succinic dehydrogenase ⁷	Succinic acid	Fumaric acid, H	Cozymase I
Triosephosphate dehydrogenase ⁸	Triosephosphate	Phosphoglyceric acid, H	Cozymase I
<i>Aerobic dehydrogenases⁶ and oxidases</i>			
			PROSTHETIC RADICAL
<i>Amine oxidases</i>			
Monoamine oxidase (tyramine oxidase) ⁷	Monoamines	Aldehydes, NH ₃ , H ₂ O ₂	
Diamine oxidase (histaminase) ⁸	Histamine, diamines	Aldehyde, NH ₃ , H ₂ O ₂	Flavin-adenine dinucleotide
<i>d</i> -Amino acid oxidase ⁹	<i>d</i> -Amino acids	Keto acids, NH ₃ , H ₂ O ₂	
<i>l</i> -Amino acid oxidase ¹⁰	<i>l</i> -Amino acids	Keto acids, NH ₃ , H ₂ O ₂	
Ascorbic acid oxidase ¹¹	Ascorbic acid	Dehydroascorbic acid, H ₂ O ₂	Cu-complex
Choline oxidase ¹⁰	Choline	Betaine aldehyde, H ₂ O ₂	
Cytochrome oxidase (indophenol oxidase, respiratory enzyme) ¹²	Reduced cytochrome	Oxidized cytochrome, H ₂ O	Fe-porphyrin complex
Glucose oxidase (penicillin B)	Glucose	Gluconic acid, H ₂ O ₂	
Glycine oxidase	Glycine	Glyoxylic acid, NH ₃ , H ₂ O ₂	Flavin-adenine dinucleotide
Histidase	Histamine, histidine	Opens ring, NH ₃	
Kynureninase	Kynurenine	Anthranilic acid	
Lipoxidase	9,10- <i>cis</i> Unsaturated fatty acids	Fatty acid peroxides	

TABLE 20 (Cont.)
OXIDIZING AND REDUCING ENZYMES

	SUBSTRATE	REACTION PRODUCTS ¹	PROSTHETIC RADICAL
Luciferase	Luciferin	Oxyluciferin	
Phenol oxidases ^{10, 12}			
Monophenol oxidase (tyrosinase) ¹⁴	Monohydric phenols	Quinones, H ₂ O	Cu-complex
Polyphenol oxidase (laccase) ⁹	<i>o</i> -Polyphenols	Quinones, H ₂ O	Cu-complex
Dopaoxidase (dopase) ¹¹	3,4-Dioxyphenylalanine	Melanin, H ₂ O	
Purine oxidases			
Allantoicase	Allantoic acid	Glyoxylic acid, urea	
Allantoinase	Allantoin	Allantoic acid	
Uricase ¹⁰	Uric acid	Allantoin, CO ₂ , H ₂ O ₂	
Xanthine oxidase (al- dehyde dehydroge- nase, Schardinger enzyme) ¹⁴	Aldehydes; hypoxanthine, xanthine	Acids; xanthine, uric acid, H or H ₂ O ₂	Flavin-adenine dinucleo- tide
Peroxidases ¹⁶	Phenols, H ₂ O ₂	Quinones, H ₂ O	Fe-porphyrin complex
Cytochrome <i>c</i> peroxi- dase	Cytochrome <i>c</i> , H ₂ O ₂	Oxidized cytochrome <i>c</i> , H ₂ O	
Tryptophanpyrrolase	Tryptophane	Kynurenine	
<i>Accessory enzymes</i>			
Acetoacetic decarboxy- lase	Acetoacetic acid	Acetone, CO ₂	
Aldolase (zymohexase)	Hexosephosphates	Triosephosphates, triose	
Aspartase	Aspartic acid	Fumaric acid, NH ₃	
Carbonic anhydrase ¹⁷	Carbonic acid	CO ₂ , H ₂ O	
Carboxylase	α -Keto acids	Aldehydes, CO ₂	Cocarboxylase, Mg
Catalase ¹¹	Hydrogen peroxide	Oxygen, H ₂ O	
Cysteic decarboxylase ¹⁰	Cysteic acid	Taurine, CO ₂	
Cytochrome <i>c</i> reduc- tase ¹⁸	Reduced cozymase II	Oxidized cozymase II, H ₂ O ₂	Flavin nucleotide
β -Decarboxylase	Oxaloacetic acid	Pyruvic acid, CO ₂	
Desulfurase	Cysteine	Pyruvic acid, NH ₃ , H ₂ S	
Diaphorase (coenzyme factor)	Reduced cozymase I	Oxidized cozymase, H ₂ O ₂	Flavin-adenine dinucleo- tide
Dopa decarboxylase ¹⁰	3,4-Dihydroxyphenyl- alanine	Hydroxytyramine, CO ₂	
Enolase	Phosphoglyceric acid	Phosphopyruvic acid, H ₂ O	Mg, Mn, Zn
Fumarase ²	Fumaric acid, H ₂ O	Malic acid	
Glucophosphomutase (isomerase)	Hexosephosphates	Isomeric hexosephos- phates	Mg, Mn
Glyoxalase	Methylglyoxal, H ₂ O	Lactic acid	—SH compounds
Histidine decarboxy- lase ¹¹	Histidine	Histamine, CO ₂	
Lysine decarboxylase	Lysine	Cadaverine, CO ₂	
Mutase (aldehydemu- tase)	Aldehydes	Acid, alcohol	Cozymase I
Myokinase	Adenosine diphosphate	Adenosine triphosphate, adenylic acid	
Oxaloacetic carboxylase	Pyruvic acid, CO ₂	Oxaloacetic acid	Mn
Transaminases (amino- phases)	Amino acids, α -Keto acids	α -Keto acids, amino acids	
Tyrosine decarboxylase	Tyrosine	Tyramine, CO ₂	

CARRIERS	ACCEPTS	DONATES
<i>Coenzyme Carriers</i>		
Diphosphothiamine (cocarboxylase)	H from pyruvic dehydrogenase	
Flavoproteins (yellow enzymes)		
Cytoflavin (flavin nucleotide) . .	H from cozymases	H to cytochrome or oxygen
Flavin-adenine dinucleotide . .	H from cozymases or special oxidases	H to cytochrome
Iron-porphyrin complexes		
Cytochromes a, b, and c	Electrons from cytochrome reductase and diaphorase	Electrons to cytochrome oxidase
Pyridine nucleotides (cozymases)	H from dehydrogenases	H to diaphorase and metabolic intermediates
<i>Carriers of Undetermined Function</i>		
Dehydroascorbic acid-ascorbic acid	H from sulfhydryl compounds	H to Cu-complexes, biliverdin, quinones
Adrenochrome-adrenaline	H from ascorbic acid and glutathione	H to cytochrome
Sulfhydryl compounds		
Oxidized glutathione-reduced glutathione	H from tissue proteins	H to Fe-complexes, ascorbic acid, adrenochrome
o-Quinones	H from cozymases	H to O via phenol oxidases

¹ H indicates activated hydrogen; O represents activated oxygen.

² Inhibited by dilute iodoacetate.

³ Inhibited by morphine.

⁴ Purine deaminases are often classified as hydrolytic enzymes. They are tentatively classified as dehydrogenases, analogous to the deaminating amino acid dehydrogenases.

⁵ Inhibited by pyrophosphate and ribonuclease.

⁶ Dehydrogenases which can react with molecular oxygen should be included with the oxidases. These enzymes transfer food hydrogen to molecular oxygen.

⁷ Inhibited by benzedrine and ephedrine.

⁸ Inhibited by Ca^{++} , CN^- , choline, ephedrine, guanidine and thiamin.

⁹ Inhibited by —SH compounds.

¹⁰ Inhibited by CN^- .

¹¹ Inhibited by —SH compounds and by CN^- .

¹² Inhibited by azide, carbon monoxide, CN^- , —SH and ribonuclease.

¹³ Inhibited by azide, *p*-aminobenzoate, carbon monoxide, and sulfonamides.

¹⁴ Inhibited by CN^- , diethyldithiocarbamate, ethylxanthate and —SH.

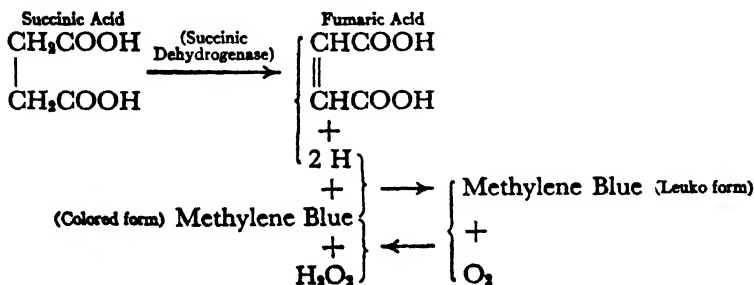
¹⁵ Inhibited by *p*-aminophenol and CN^- .

¹⁶ Inhibited by azide, CN^- , and —SH.

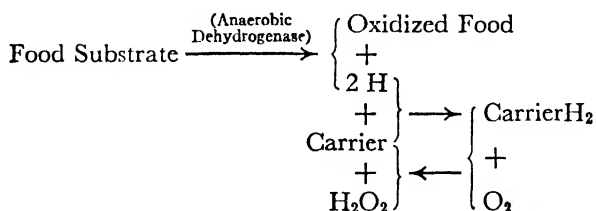
¹⁷ Inhibited by CN^- , CNS^- , —SH, and sulfanilamide.

¹⁸ Inhibited by ribonuclease.

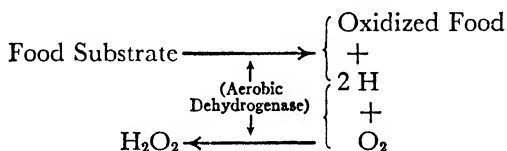
form, and either hydrogen peroxide or water is formed. With succinic acid as substrate, the oxidation proceeds as follows:



This is the equivalent of a general type of biological oxidation in which one or more physiological bridging systems act as coenzymes to anaerobic dehydrogenases. The type diagram would be:



There is a group of *aerobic dehydrogenases* (*d*-amino acid, *l*-amino acid, ascorbic acid, choline, glucose, glycine, monoamine and xanthine oxidases, and uricase) which not only activate the hydrogen of their substrates but also transfer it to molecular oxygen with production of hydrogen peroxide. The type diagram for this reaction would be:



Superficially, the action of aerobic dehydrogenases appears to constitute a simpler type of biological oxidation since only the food substance, specific dehydrogenase, and molecular oxygen are required. However, aerobic dehydrogenases contain firmly bound prosthetic carrier radicals similar to the free carriers which associate with anaerobic dehydrogenases as easily dissociated complexes. The action of both types of dehydrogenases is, therefore, fundamentally similar; aerobic dehydrogenases merely consist of a single conjugated molecule instead of separable units. It has been demonstrated that amino acid, glucose (penicillin B), glycine and xanthine oxidases react by virtue of flavoprotein carriers which constitute prosthetic radicals of these dehydrogenases. The reduced forms of flavoprotein carriers (yellow enzymes) can react with molecular oxygen *in vitro*, but in living tissues most of the oxygen does not combine directly with hydrogen carriers.

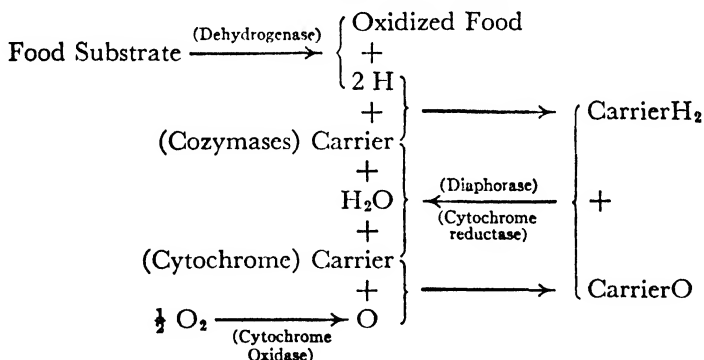
OXIDASES

Aerobic oxidation by anaerobic dehydrogenases requires the assistance of another group of enzymes, the oxidases, which activate molecular oxygen and render it available for combination, indirectly, with the hydrogen liberated by dehydrogenases. In the absence of oxidases, many dehydrogenase reactions cease as soon as the hydrogen acceptors are saturated; and the energy sources of living tissue are greatly curtailed, inasmuch as the combination of oxygen with hydrogen is the major calori-

genic reaction. Oxidases are highly auto-oxidizable enzymes; they form active oxygen (not hydrogen peroxide), and cannot function anaerobically. Certain oxidases are compounds of protein with iron-porphyrin complexes or copper complexes (pages 534 and 536). Such oxidases are inactivated or inhibited by azide, carbon monoxide, cyanide, and sulfide because these substances combine with the metal atoms of the prosthetic radicals.

Inhibition of oxidases by cyanide ions explains why cyanides are rapidly fatal to animals. Carbon monoxide, on the other hand, causes death before it seriously affects cell oxidases. A pressure of one atmosphere of this gas does inhibit cell respiration by combining with cytochrome oxidase, but even a few tenths of 1 per cent of carbon monoxide in the inspired air is sufficient to displace the oxygen from oxyhemoglobin. Death, therefore, results from suffocation caused by the inability of the erythrocytic carbon monoxide-hemoglobin to transport the necessary oxygen to the tissues. Carbon monoxide, cyanide, and sulfide poisonings are discussed in greater detail on pages 556 to 558.

Common aerobic oxidations which operate through oxidases require the following factors: food substrate, specific dehydrogenase, hydrogen carrier, flavoprotein (cytochrome reductase, diaphorase), cytochrome, oxidase, and oxygen. The type diagram for such reactions is as follows:



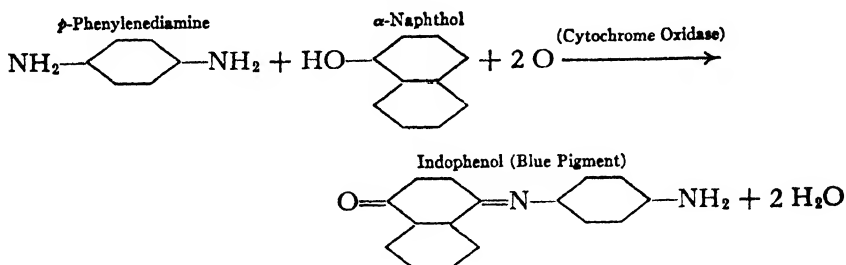
(Activated oxygen does not actually combine with cytochrome; instead, electrons are transferred in reverse direction.)

Note that water, rather than hydrogen peroxide, is formed in this reaction. The cytochrome reductase and the diaphorase or "coenzyme factor" indicated in the diagram are flavoprotein enzymes which catalyze the transfer of electrons between hydrogen carriers and oxidized cytochrome. The prosthetic radical of diaphorase is a flavin-adenine dinucleotide, while cytochrome reductase contains a flavin mononucleotide radical (page 104).

Cytochrome Oxidase

This enzyme, sometimes termed the respiratory enzyme of Warburg, is present in many cells. It was formerly known as indophenol oxidase

because of its supposed direct action on a mixture of α -naphthol, *p*-phenylenediamine, and molecular oxygen:



It has been shown that cytochrome oxidase oxidizes only cytochrome, and the oxidized form of the latter is responsible for the oxidation of the indophenol reagent.

Cytochrome oxidase is destroyed in tissues by heating above 60° C., or by drying or freezing. This enzyme has, as prosthetic radical, an auto-oxidizable iron-porphyrin complex (a pheohemin) which can be inactivated by azide, ribonuclease, hydrogen sulfide, cyanide ions, or by carbon monoxide in the dark. Its chief biological function is the activation of oxygen. In mammals, molecular oxygen diffuses into the tissues from transported oxyhemoglobin and reacts with cytochrome oxidase which activates it and accepts electrons from cytochrome. The latter is an important carrier which is present in many cells. Cytochrome oxidase is found in largest concentrations in the heart, kidney, brain, and muscle; the intestine and fetal tissues contain only small quantities, and early embryonic tissue has none. Cytochrome oxidase activity is low in tumors. Adrenalectomy leads to a decrease of this activity in heart, kidney, and liver. Deficiency of copper in the diet greatly decreases the cytochrome oxidase in mammalian tissue.

Phenol Oxidases

The prosthetic radicals of these enzymatic proteins contain from 0.2 to 0.34 per cent copper (crystalline tyrosinase contains 0.25 per cent). The phenol oxidases are inhibited by azide, carbon monoxide, cyanide, and sulfide, and also by diethyldithiocarbamate, ethylxanthate, sulfonamides, and *p*-aminobenzoate. Some of these enzymes are very sensitive to carbon monoxide, and they are not reactivated by light. The phenol oxidases activate oxygen and convert phenols to quinones. The cupric atom of phenol oxidases is probably reduced to the cuprous form by the substrate, and the copper atom is reoxidized by molecular oxygen. In the case of dopaoxidase (Table 20, page 94) the quinones formed can undergo condensation to produce the dark-colored pigments (melanins) of hair, iris,

skin, and the like. The chemistry of melanin formation is discussed on page 375. Dopaoxidase is found in the melanoblasts of the epidermis of most higher vertebrates, but is absent from albinos. The *o*-quinones formed by the phenol oxidases give the guaiac test, and can be reduced to phenols in the presence of sulfhydryl compounds. They also oxidize ascorbic acid and the dihydrocozymases; the catechol formed from *o*-quinone in these reactions can be reoxidized by polyphenol oxidase. Hence, the polyphenol oxidase-phenol system can act as a hydrogen carrier with cozymase-determined dehydrogenases and peroxidases.

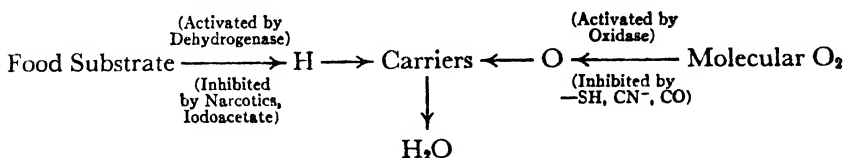
Peroxidases and Catalase

These widely distributed enzymatic proteins have iron-porphyrin prosthetic radicals; they are inhibited by azides, cyanides, sulfides, sulfonamides, and thiourea but not by carbon monoxide. As a class, they liberate oxygen from hydrogen peroxide. The active oxygen formed by peroxidases can be utilized for the oxidation of aromatic amines, diamines, ascorbic acid, reduced cytochrome *c*, and phenols, as for example, the oxidation of guaiaconic acid to guaiac blue in the well-known guaiac test (page 530). A variety of biological oxidations give products similar to those formed more slowly from the same substrates by the action of hydrogen peroxide *in vitro*. Peroxidases may, therefore, be considered as possible factors in biological oxidation, but their physiological role is undetermined. Leukocytes contain a green verdoperoxidase, and other peroxidases have been found in eggs, milk, adrenal gland, and chick embryo. Peroxidase II, which has been obtained in crystalline form from horseradish, combines with hydrogen peroxide to form a green compound. Its activity is inhibited by excess hydrogen peroxide, and its iron atom appears to remain in the ferric state during activity. Cytochrome *c* peroxidase acts specifically on cytochrome.

The oxygen formed from hydrogen peroxide by catalase is not utilized by any known dehydrogenase-carrier system. Hence, catalase is not an oxidase but rather an accessory enzyme which decomposes the hydrogen peroxide formed by the action of aerobic dehydrogenases or by the reaction of certain carriers with molecular oxygen. Appreciable concentrations of hydrogen peroxide are antiseptic and toxic to living cells. Crystalline catalase is a protein-hematin compound; its iron atoms combine with hydrogen peroxide which is decomposed to water and oxygen. The largest concentrations of catalase are found in the liver and erythrocytes (approximately 50 and 150 mg. per cent, respectively). The catalase activity of liver and kidney decreases in copper or iron deficiency; hepatic catalase is low in fetal liver and in hepatomas, as well as with malignancy elsewhere in the body.

BRIDGING SYSTEMS (ELECTRON TRANSPORTERS, CARRIERS, OR HYDROGEN AND OXYGEN ACCEPTORS)

The physiological carriers listed in Table 20, page 95, have properties similar to those of the oxidation-reduction indicators. They act as co-enzymes to dehydrogenases and oxidases:



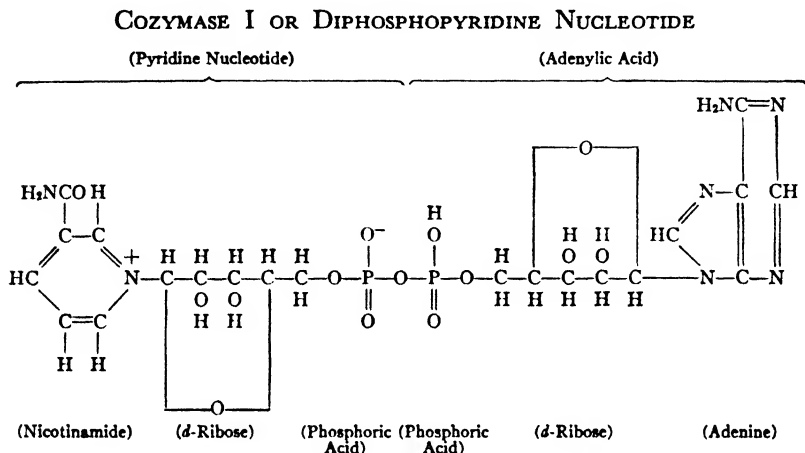
The potential energy of oxidation-reduction systems is made available for physiological processes by these bridging systems, which transfer electrons or activated hydrogen to specific chemical acceptors of living cells.

Cytochromes

The most widely distributed oxygen carriers of aerobic cells are the cytochromes *a*, *b*, and *c*. These compounds of protein with iron-porphyrin complexes (Table 90, page 527) can be detected, spectroscopically, in living cells. The reduced form of cytochrome *c*, the most stable and best studied member of the group, has characteristic absorption bands in the green portion of the spectrum (Table 91, page 529). The bands become permanent in cells whose cytochrome oxidase is inhibited by cyanide, sulfide, or carbon monoxide. The bands disappear, and reduced cytochrome *c* is no longer formed, when cellular dehydrogenases are inhibited by narcotics. Hence, cytochrome oxidase is essential for the transfer of electrons from cytochrome, and dehydrogenase and cytochrome reductase are necessary for the reduction of oxidized cytochrome in living cells. Cytochrome *c* is reduced *in vitro* by adrenaline, ascorbic acid, or cysteine. The major portion of the oxygen utilization by living yeast cells and by a number of animal tissues requires cytochrome carriers. The largest quantities of cytochrome are found in cardiac and skeletal muscles; intermediate quantities are found in kidney and liver; and smaller quantities are present in other glands, embryonic tissue, brain, and lung. Very little is present in malignant tumors (about 1.5 mg. per cent); hepatic tumors contain much less cytochrome *c* than normal liver. Adrenalectomy leads to a decrease of renal and hepatic cytochrome *c*. Highly aerobic bacteria have a complete cytochrome oxidase-cytochrome system, while the facultative anaerobes are deficient in one or more of the components (usually cytochrome *c*); strict anaerobes, such as *Clostridia* and certain streptococci, contain neither cytochrome nor cytochrome oxidase.

Cozymases, Codehydrogenases, or Pyridine Nucleotides

These important substances are widely distributed hydrogen acceptors. They contain two molecules of *d*-ribose, one each of adenine and nicotinamide, and several molecules of phosphoric acid. *Cozymase I* or *diphosphopyridine nucleotide* has the following structure:



The formula for *cozymase II* or *triphosphopyridine nucleotide* differs only in the presence of an extra phosphoric acid radical linked to one of the phosphoric radicals in cozymase I.

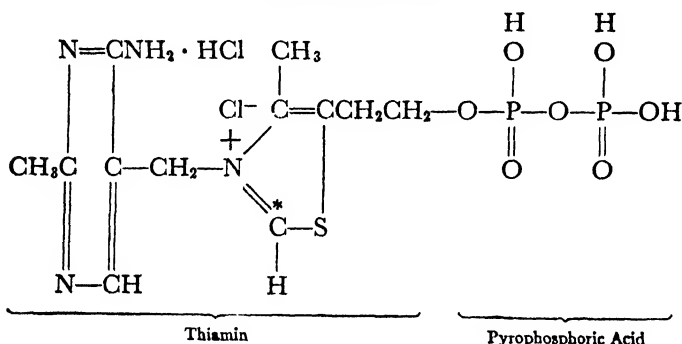
The unsaturated bond at the nitrogen atom of nicotinamide allows hydrogen to be accepted reversibly, and is responsible for the carrier activity of the cozymases. Nicotinamide is a vitamin of the B group (page 659). In man it has antipellagric properties, and is necessary for maintenance of the normal metabolism of the nervous system, skin, and mucous membranes. Cozymases are found in largest concentration in liver (120 mg. per cent), kidney (100 mg. per cent), retina, gray matter of the brain, muscle (50 mg. per cent), and the adrenal cortex. Cozymase concentration is low in malignant tumors, leukemia cells, and embryonic tissues. Human blood contains from 2 to 3.5 mg. per cent of total cozymases, present entirely in the erythrocytes. These cells have approximately 2 mg. per cent cozymase I and 4 mg. per cent cozymase II. In nicotinic acid deficiency, the cozymase content of liver and muscles decreases, while that of the brain, kidney, and blood is maintained. Reduced cozymases are oxidized by *o*-quinones, but not by molecular oxygen; they accept hydrogen from dehydrogenases and transfer it to flavoproteins and metabolic intermediates. These carriers function only in the presence of specific dehydrogenases. Further details of the functions of cozymases and adenylic acid in carbohydrate catabolism are given on pages 323 and 319, respec-

tively. The cozymases are destroyed by ultraviolet light. Nucleases hydrolyze the cozymases to pyridine nucleotide and adenylic acid.

Cocarboxylase, Diphosphothiamin, or Thiaminpyrophosphate

This carrier is similar in action to the cozymases except that it is a specific prosthetic radical for pyruvic acid dehydrogenase and carboxylase, and its action is, therefore, limited to oxidation of carbohydrates. It is widely distributed in cells, the largest amounts being present in liver and kidney (about 500 γ per cent); brain has 300 γ per cent. Normal human blood contains 10 ± 3 γ per cent of cocarboxylase, contained entirely within the cells. (For the origin of the name cocarboxylase see page 107.)

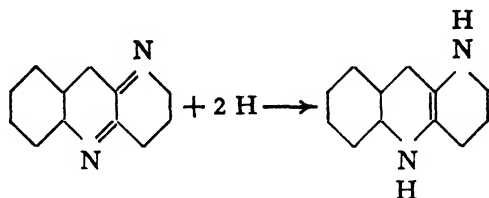
COCARBOXYLASE



The thiamin, or vitamin B₁, portion of the cocarboxylase molecule contains an unsaturated bond (marked with an asterisk) which functions as the hydrogen acceptor. In thiamin deficiency there is an accumulation of the intermediate products of carbohydrate metabolism, lactic and pyruvic acids, and the syndrome of beriberi develops (page 655). Under these circumstances the cocarboxylase of blood and tissues is decreased. (See page 324 for further discussion of cocarboxylase.)

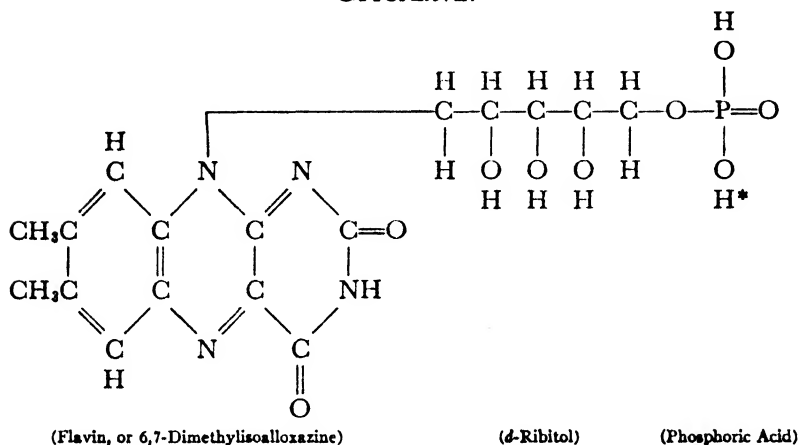
Flavin Nucleotides and Flavoproteins

The most important function of these hydrogen carriers is to bridge cytochrome and the hydrogen acceptors discussed previously. Riboflavin or vitamin B₂ is an orange-colored flavin, lyochrome or isoalloxazine pigment, which is widely distributed in living tissues, particularly in anaerobic cells. Solutions of riboflavin show a yellow-green fluorescence; in neutral solution the ribitol is split off by light, while in alkaline solution riboflavin decomposes to lumiflavin. Here, again, unsaturated bonds allow the reversible reduction of the pigment to a colorless or leuko form. The hydrogen is added as follows:



In living cells the riboflavin is esterified with phosphoric acid to produce the flavin nucleotide, *cytoflavin*, which has the following structure:

CYTOFLAVIN



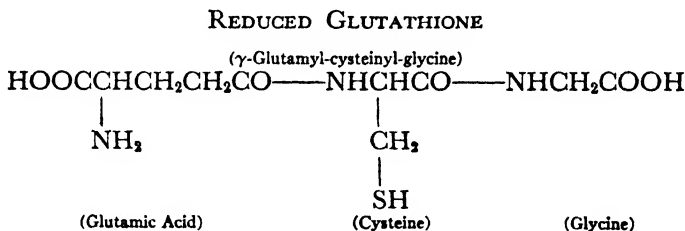
Through the phosphoric acid radical, flavin nucleotide can readily unite with dehydrogenases to form the easily dissociable *flavoproteins* or *yellow enzymes*; and in all tissues except the retina, riboflavin exists chiefly as these compounds. The growth-promoting properties of vitamin B₂ are very probably connected with the coenzymic functions of the flavoproteins, which act as important carriers in the catabolism of carbohydrates and amino acids. The flavoprotein oxidases differ from cytochrome oxidase in their ability to form hydrogen peroxide. Their activity is inhibited by *p*-aminophenol, cyanide, iodoacetate and sulfide.

The flavoprotein, *diaphorase* or *coenzyme factor*, is widely distributed in animal, bacterial, and plant tissues. Its prosthetic radical consists of cytoflavin and a molecule of adenylic acid united at the position marked with an asterisk in the formula given above. This green-yellow nucleotide, called *flavin-adenine dinucleotide*, therefore has the same general structure as cozymase I except that a riboflavin unit replaces the nicotinamide and *d*-ribose units. The flavin-adenine dinucleotide radical combines with a specific protein to form diaphorase; it can also combine with other proteins (dehydrogenases) to produce the enzymes known as

d-amino acid oxidase, glycine oxidase, and xanthine oxidase. Riboflavin deficiency decreases the *d*-amino acid oxidase activity of the liver and kidney, and the xanthine oxidase activity of the liver; these enzymes are also subnormal in hepatic tumors and fetal liver. Other flavoprotein enzymes include *l*-amino acid oxidase, glucose oxidase (penicillin B, page 479), and *cytochrome reductase*; the latter has a cytoflavin prosthetic radical. The simpler flavoproteins can accept hydrogen from cozymases and transfer it to molecular oxygen, but in living cells diaphorase and cytochrome reductase improve upon this somewhat sluggish oxidation. They accept hydrogen specifically from cozymases and transfer it to the cytochrome system. When the latter is reoxidized by cytochrome oxidase, the final physiological oxidation product, water, is formed. (See diagram on page 97.) There are apparently two forms of cytochrome reductase, which accelerate transfer of electrons between the two cozymases and cytochrome.

Glutathione

This sulfhydryl compound is a tripeptide containing the amino acids, cysteine, glutamic acid, and glycine. It is widely distributed in cells, where it occurs chiefly in the reduced form.



The —SH or sulfhydryl radical is the portion of the molecule which acts as reductant. In the presence of alkali and traces of iron cations, copper cations, or iron-porphyrin compounds, the hydrogen atom of this radical can be transferred to molecular oxygen. Two glutathione residues then combine to form the disulfide or oxidized form of glutathione, which contains the —S—S— linkage. Oxidized glutathione can, in turn, be reduced by hydrogen to form the original tripeptide. The reaction occurs in tissues but is not enzymatic, because tissues whose enzymes have been destroyed by heat can still reduce oxidized glutathione. The —SH radicals of tissue proteins are responsible for the reduction.

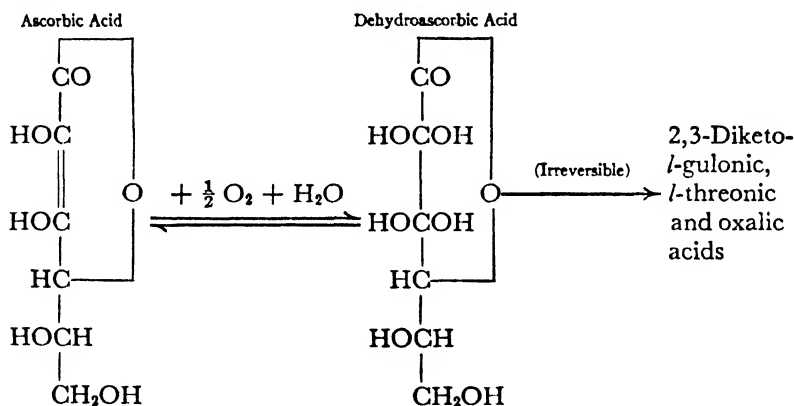
Glutathione activates certain proteolytic and protein-synthesizing enzymes (cathepsins), and thus accelerates protein synthesis and growth. It also aids in keeping the —SH radicals of dehydrogenase proteins in active reduced forms. Iodoacetate, maleic acid, and heavy metals inhibit dehydrogenases, phosphorylases, certain proteinases, and the like, by

combining with their sulfhydryl radicals; previous addition of reduced glutathione, cysteine, or thiosulfate can prevent these effects.

No known dehydrogenase uses glutathione as hydrogen acceptor. Only in the case of the accessory enzyme, glyoxalase (Table 20, page 94), is glutathione to be regarded as a coenzyme. The fact that lactic acid accumulates in tissues under anaerobic conditions and disappears with increased oxygen tension may have some relation to the reduction and oxidation of glutathione. However, investigations to date do not confirm the supposed importance of glyoxalase in lactic acid formation. A more significant function of reduced glutathione is to maintain vitamin C in its reduced form in tissues.

Ascorbic Acid (Vitamin C)

This vitamin is a constituent of many tissues, and is especially concentrated in the adrenal gland. Ascorbic acid can be oxidized reversibly to dehydroascorbic acid, as shown in the following equation:



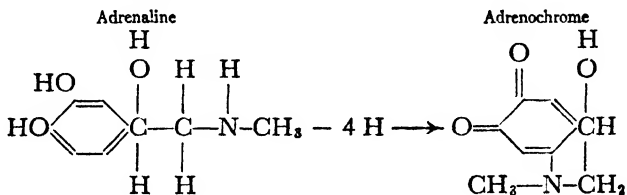
Above pH 4.0 irreversible oxidation occurs, as shown in the equation. Below pH 7.6 traces of copper cations, or exposure to light, catalyze the oxidation of ascorbic acid; metaphosphates and pyrophosphates inhibit the oxidation. Ascorbic acid is oxidized by the cytochrome oxidase-cytochrome system, and by ascorbic oxidase, a common constituent of plants.

Tissue vitamin C is kept in the reduced state chiefly by glutathione and the sulfhydryl radicals of proteins, and is thus protected from irreversible oxidation. Ascorbic acid is active in reducing the bile pigment, biliverdin, to bilirubin, and oxyhemoglobin to choleglobin, in tissues. It also reduces quinones, inhibits the action of dopaoxidase and at times decreases the melanotic pigmentation of Addison's disease. At present, no definite role in biological oxidation can be assigned to ascorbic acid; it is not an accep-

tor for any known dehydrogenase. Together with glutathione, it appears to be necessary for the normal metabolism of the lens. Ascorbic acid deficiency produces scurvy. Tissue respiration of scorbutic animals is stimulated by administered ascorbic acid. (See also page 667.)

Adrenaline and Adrenochrome

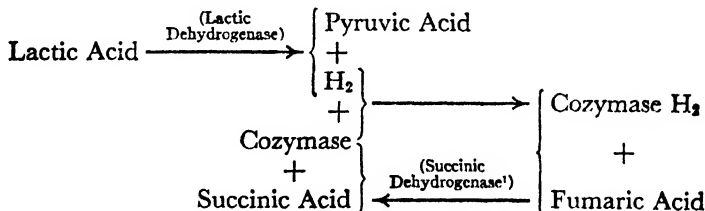
Adrenaline, a hormone of the adrenal medulla, is readily oxidized to an unstable red indol compound, adrenochrome:



Adrenochrome is rapidly formed by the action of the cytochrome oxidase-cytochrome system and by polyphenol oxidase; ascorbic acid and glutathione protect adrenaline from such oxidation. A few anaerobic dehydrogenase-cozymase systems can use adrenochrome in place of flavoprotein as hydrogen acceptor, but the action is sluggish and may not occur physiologically.

Coenzymic Metabolic Intermediates

The foregoing brief review of carrier activity may be summarized by the statement that cozymases, cocarboxylase, flavoproteins, and cytochromes are proved coenzymic hydrogen and electron acceptors in biological oxidations; while glutathione, ascorbic acid, and adrenaline are apparently of greater concern in non-enzymatic reducing reactions. The list of hydrogen acceptors should also include intermediate oxidation products of foods (especially α -keto acids), which can serve as additional physiological carriers in *linked dehydrogenase systems*. This action is accomplished by reversal of the activity of one of the dehydrogenases, as illustrated by the following example:



¹ Reverse action of the dehydrogenase.

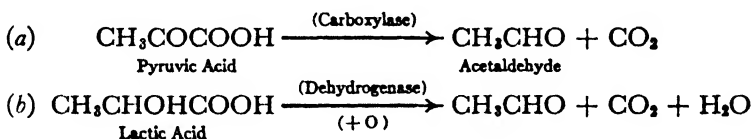
Lactic acid is oxidized anaerobically to pyruvic acid while fumaric acid (an intermediate) is reduced to succinic acid; the latter can then be reconverted to fumaric acid by the ordinary action of succinic dehydrogenase in co-operation with the cytochrome-cytochrome oxidase system.

Glutamic acid can function as a hydrogen carrier in somewhat similar fashion; its dehydrogenase can operate with either of the cozymases. Certain amino acids act as hydrogen acceptors in the metabolism of proteolytic anaerobic bacteria. The energy for the growth and development of these organisms is furnished by transfer of protons from alanine, leucine, phenylalanine, or valine to arginine, glycine, hydroxyproline, ornithine, or proline.

ACCESSORY ENZYMES

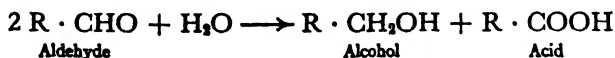
This group (Table 20, page 94) includes enzymes which hydrate, dehydrate, or remove fragments of food molecules, or rearrange their structure. As a matter of convenience, catalase and diaphorase are also placed in this group. The action of other accessory enzymes will be considered elsewhere in the text.

Carboxylase is defined as an enzyme which acts directly on carboxyl radicals of α -keto acids (as for example, pyruvic acid) to release carbon dioxide. There are, however, two possible mechanisms of decarboxylation, namely, (a) direct fragmentation, defined as decarboxylation or true carboxylase action, and (b) an oxidative decarboxylation of the keto acid or corresponding hydroxy acid:



Ordinary lactic dehydrogenase converts lactic acid to pyruvic acid, but the two acids are reversibly interconvertible in animals, and they may form a linked system. In animal tissues, decarboxylation of pyruvic acid is chiefly oxidative, and cocarboxylase is regarded as a coenzyme of a pyruvic acid dehydrogenase. Purification of this dehydrogenase and carboxylase should allow clarification of the details of decarboxylation.

The enzyme, *mutase*, catalyzes the Cannizzaro reaction of aldehydes:



This simultaneous oxidation and reduction of two aldehyde molecules may be dependent upon linked reactions.

ENERGY METABOLISM

"All scientific work implies that there is an order in nature, which we can understand by the use of reason, but it is there whether we understand it or not." — MORRIS R. COHEN

CALORIC VALUES

The important energy sources of the body are carbohydrate, fat, and protein foods. The energy evolved during biological oxidation of the non-protein food materials is equal to that measured in a bomb calorimeter. In the human body, the nitrogen of proteins is largely oxidized to urea; whereas in the bomb calorimeter, it is transformed into nitric acid with the liberation of an extra 1.2 calories per gram of protein. The heat produced by the oxidation of any given food can be calculated either from its oxygen consumption or from carbon dioxide production.

The accepted physiological caloric values¹ of foods are given in Table 21. The value for carbohydrate food is a selected average since starch yields 4.23 calories, disaccharides 3.95 calories, and monosaccharides (glucose) 3.75 calories per gram. The accepted caloric value for food fat is an average for animal, vegetable, and butter fats. Vegetable proteins give 3.95 calories per gram, and the casein of milk yields 4.4 calories per gram. The average caloric value selected for protein food is slightly lower than that of meat proteins; it represents a dietary mixture of three parts of animal protein to two parts of vegetable protein. It is obvious from these considerations that calculated physiological energy output is subject to slight errors.

RESPIRATORY QUOTIENT

The respiratory quotient (R.Q.) is defined as the volume of expired carbon dioxide divided by the volume of oxygen inspired

$$\frac{\text{CO}_2}{\text{O}_2}$$

The values in Table 21 show that this quotient is characteristic for different foods. Oxidation of fat lowers the respiratory quotient, whereas carbohydrate oxidation raises it. The respiratory quotient can be used to calculate the composition of the food mixture undergoing oxidation in the animal. However, there are other factors which influence the respiratory quotient. The physiological synthesis of fats from carbohydrates raises it, because oxygen from the carbohydrate is available for tissue oxidations and decreases the oxygen intake in the lungs. Changes in acid-base balance also affect the quotient by influencing the rate of carbon dioxide excretion.

¹ In biological calorimetry the calorie employed is the large or kilogram calorie, which is the heat required to raise 1 kg. of water from 15° to 16° C.

When either fructose or sucrose is eaten, unusually large amounts of lactic acid (an intermediate carbohydrate metabolite) are liberated. The lactic acid frees carbon dioxide from the alkali reserve and rapidly raises the respiratory quotient. The fact that foods have different respiratory quotients necessitates the use of caloric equivalents for oxygen and carbon dioxide which are specific for each type of food. (See the last two columns of Table 21.)

TABLE 21
CALORIC VALUE OF FOODS

	AVERAGE CALORIES PER GM.	R.Q. (CO ₂ /O ₂)	CALORIES PER LITER	
			O ₂	CO ₂
Carbohydrate	4.1	1.00	5.05	5.05
Protein	4.1	0.80	4.48	5.58
Fat	9.3	0.71	4.69	6.63
Protein in total diabetic	1.25	0.63	3.82	6.46
Fat in total diabetic . .	7.15	0.66	4.61	7.01
Lactic acid	3.6	1.00	4.85	
Acetoacetic acid		1.00		
β-Hydroxybutyric acid .		0.89		
Glycerol	4.55	0.86		
Acetone		0.75		
Ethyl alcohol	7.0	0.67	4.85	

One gram of urinary nitrogen corresponds to the oxidation of 6.25 gm. of protein, an exchange of 5.91 liters of oxygen and 4.76 liters of carbon dioxide, and the liberation of approximately 26.5 calories.

METHODS OF BIOLOGICAL CALORIMETRY

Physiological energy production can be determined by either direct or indirect calorimetry. In *direct calorimetry* the actual caloric output of the living organism is measured in a carefully insulated air-tight chamber. Various devices are necessary to determine the temperature changes and the heat eliminated from the body through vaporization of water and radiation. This elaborate method is not practical for clinical use because it entails considerable expense and is not comfortable for all patients.

The method of *indirect calorimetry* is used for ordinary clinical work. It depends on estimations of oxygen consumption and carbon dioxide elimination from which the energy output is calculated. Indirect calorimetry has been shown to be capable of yielding results which are more than 99 per cent accurate. Since the three main classes of foodstuffs are oxidized simultaneously, oxygen and carbon dioxide determinations provide insufficient data for calculating the composition of the catabolic mixture and the exact caloric output. The nitrogen content of the urine

secreted during the metabolic period must also be known, for it is a convenient approximate measure of the amount of protein catabolized. That portion of the oxygen and carbon dioxide exchange which is of protein origin can be calculated from the urinary nitrogen. With the values of Table 21, the outline of the calculations for indirect calorimetry is as follows:

$$\text{Grams urine nitrogen} \times 5.91 = X \text{ liters } O_2$$

$$\text{Grams urine nitrogen} \times 4.76 = Y \text{ liters } CO_2$$

$$\frac{\text{Total } CO_2 - Y}{\text{Total } O_2 - X} = \text{non-protein R.Q.}$$

$$\text{Protein calories (P)} = X \times 4.48$$

$$\text{Non-protein calories (N)} =$$

$$\left(4.69 + \frac{\text{non-protein R.Q.} - 0.707}{0.293} \right) \times 0.361 \times (\text{Total } O_2 - X)$$

Under normal conditions, the urinary nitrogen excreted during a twenty-four-hour period is a fairly accurate measure of the protein catabolism. With sudden changes in the protein content of the diet this is no longer true, since there is a lag period of approximately three days before the new nitrogen equilibrium is established (page 422). Another factor which affects nitrogen excretion is a change in kidney function associated with the rapid appearance or disappearance of renal disease or of dehydration. Many nephritic patients are unable to concentrate urine properly and, therefore, they excrete a part of their day portion of nitrogen in the night urine. Also, plasma proteins are excreted in very appreciable quantities in nephritic urine. This nitrogen must be excluded in determinations of the catabolized nitrogen of the urine. The use of urine nitrogen in short metabolic determinations is very unreliable, because there is a lag in the excretion of certain nitrogenous substances following meals containing protein.

Approximate Clinical Methods

Under sufficiently standardized conditions, approximate metabolic rates can be calculated safely from the determination of oxygen consumption. A selected average respiratory quotient (0.82), assumed to be constant under the conditions of the test, and a corresponding caloric value of 4.8 per liter of oxygen are used. Simplified portable clinical instruments have been devised for the determination of metabolism during short periods (from six to ten minutes). These instruments are of the closed circuit type, soda lime being used to absorb the expired carbon dioxide. The oxygen consumption, registered on a kymograph, is corrected for temperature and pressure variations and is converted to the basal metabolic rate by means of tables. Detailed descriptions of the instruments are available among the references cited.

Measurements of oxygen consumption are preferred to estimations of carbon dioxide excretion, because caloric calculations based on the latter are less accurate. With a changing respiratory quotient, the carbon dioxide elimination fluctuates four times as much as the oxygen consumption. Also, the excretion of carbon dioxide in respired air is much more subject to acid-base and respiratory disturbances than is the oxygen intake. An unusual increase in oxygen supply to the lungs has very little influence on the oxygen tension of blood, and practically no effect on the rate of oxidation by the tissues. For these reasons, oxygen intake is chosen as the basis for calculating energy values; carbon dioxide determinations merely assist in estimating respiratory quotients.

Precautions for Clinical Determinations

Short-period methods give satisfactory energy values, but these values are only approximate and are easily affected by temporary respiratory fluctuations. Therefore, the clinical determination of basal metabolism is most reliable when it is repeated on successive days.

Errors are easily introduced in short-period experiments by delay in the excretion of carbon dioxide, or by a sudden sweeping out of the gas. If the tension of carbon dioxide in the plasma is suddenly lowered, the plasma bicarbonate will liberate carbon dioxide and the pulmonary excretion of this gas will be increased. Nervous excitement in patients who are unaccustomed to the metabolism apparatus may result in overventilation, increased carbon dioxide excretion, and a raised respiratory quotient. Such effects can be detected by irregularities in respiration. A similar disturbance is produced during rapidly developing states of metabolic acidosis wherein carbon dioxide is liberated from the alkali reserve. Opposite conditions exist when acidosis is subsiding, or when alkalosis is developing. The consumption of oxygen does not vary greatly under these conditions, but it is markedly increased by muscular activity. Short-period indirect determinations of energy output during exercise may be quite inaccurate, because the oxygen absorption lags behind the heat output. The muscles accumulate an "oxygen debt" by anaerobic processes. Later, during the muscular recovery period, the oxygen intake exceeds the energy production.

BASAL METABOLISM

Basal metabolism is defined as the rate of energy production in the morning, approximately twelve hours after the last meal. It is measured with the patient awake, in complete mental and physical repose, and at a comfortable room temperature. Emotional stress and previous muscular exertion must be entirely avoided, and the respiration should be entirely involuntary during the determination. The technician should be alert

to detect emotional states and muscular movements, since these constitute the greatest source of error in basal metabolic determinations.

Basal metabolism is fairly constant, and it represents the minimal energy metabolism for the waking hours. During sleep, the muscles are more completely relaxed and the metabolism is from 10 to 15 per cent lower than the standard basal value. The basal metabolism varies with age, sex, and size of normal subjects. Adult males of the same age and size deviate less than 10 per cent from the standard basal rate. Both weight and height are important factors in determining body size, and the standard rates are, therefore, predicted on the basis of age, height, weight, and sex. Detailed tables of predictions are available in clinical laboratory handbooks. The basal rates for women are from 7 to 10 per cent lower than those for males of similar age and size.

Basal rates are frequently expressed as calories per square meter of body surface (which can be calculated from height and weight). The average surface areas are 1.8 square meters for men and 1.6 square meters for women. On the basis of body surface, the basal metabolism of newborn infants is near that of adults; but their respiratory quotient approaches that of pure carbohydrate combustion (1.00), whereas the average respiratory quotient for adults is 0.82. The high respiratory quotient of the newly born falls rapidly to 0.71 during the first day of life, and then rises again to approximately 0.8 by the sixth day. The basal metabolic rate per square meter of body surface increases during the first few months of life and reaches a maximum at three to four years, at which time it is from 15 to 20 per cent greater than that of the adult. A second smaller rise occurs preceding puberty; the basal metabolism then falls gradually with advancing age (Table 22).

TABLE 22

APPROXIMATE RELATION OF BASAL METABOLIC
RATE TO AGE IN ADULT MALES

BODY WEIGHT (KG.)	CALORIES PER KG. PER DAY			
	20 Years	30 to 40 Years	50 to 60 Years	70 to 80 Years
30	38	35	33	32
40	33	31	29	28
50	29	27	26	25
60	27	25	24	23
70	25	24	22	21
80	23	22	21	20
90	22	20	19	18
100	21	19	18	17
110	20	18	17	17
120	19	18	17	16

The fact that basal metabolism is more nearly proportional to the superficial surface of the body than to body weight is not necessarily due to Newton's laws of cooling. When homothermic or warm blooded animals are kept in environments at their body temperature there is no need for heat production to maintain the body temperature; nevertheless the heat production continues. The respiratory rates of tissue fragments taken from large and small animals are unequal even though the surfaces are equal. Varying rates of oxidation depend partly upon the content of non-respiring or paraplasmic substances. These inactive proteins increase with age.

Clinicians do not always evaluate basal metabolic rates in terms of calories per square meter of body surface. Many prefer to use the percentage deviation of the case from the predicted standard. Thus, if a patient is producing 33 calories per hour and his predicted standard is 30 calories per hour, he is said to have a plus 10 per cent basal metabolic rate.

Effects of Race and Climate

The effects of these factors on the metabolic rate have been disputed. A number of studies have shown approximately minus 10 per cent values for women in Florida and Oklahoma, and for Chinese women in America. The metabolic rate has also been reported low for Negroes, Filipinos, Syrians, Australian aborigines, and Oriental peoples, and for white races inhabiting the tropics. Others have found essentially normal values in Japan, Mexico, and Oklahoma. It is apparent that the low basal rates that have been reported have been found chiefly in connection with warm climates and vegetarian diets. Values of plus 30 per cent have been reported for Eskimos living in cold climates and subsisting on meat and fat diets. The clinician recognizes that 10 per cent deviations from metabolic standards have little pathological significance.

BODY TEMPERATURE AND HEAT LOSS

The heat output of living organisms depends on oxidations occurring within the cells. These oxidative processes are stimulated by muscular activity, the specific dynamic action of foods, exposure to cold, and environmental temperatures above 90° F. (32° C.). Heat is lost from the body through excreta, by convection, radiation, evaporation of water from the lungs and skin, and by warming the air. The production and dissipation of heat require delicate regulation to maintain the rather constant temperature of homothermic animals. The oral temperature of human beings is normally maintained at approximately 98.6° F. (37° C.); it falls slightly during sleep. The temperature-regulating center is in the diencephalon. The mechanism of temperature regulation involves automatic reflex control of the innervations of sweat glands and blood vessels. Heat loss is also partly controlled, in human beings, by voluntary adjustment of clothing.

Approximately three fourths of the normal heat loss in temperate climates is through radiation and convection from the skin. Evaporation from the skin accounts for another 15 per cent, and evaporation from the lungs for approximately one half this amount. At environmental temperatures above 68° F. (20° C.) physical regulation of body temperature predominates; below this temperature, chemical regulations stimulate energy production. At low temperatures, the blood vessels of the skin and respiratory passages constrict, while at high temperatures they dilate and the increased blood flow allows more effective cooling. Exposure to cold incites chemical regulation, which involves increased involuntary activity of skeletal muscle. Shivering commences at skin temperatures of approximately 66° F. (19° C.) but voluntary muscular action can replace it as a method of increasing the energy output. When exposure continues for some length of time the thyroid gland is stimulated; hormones set free from this gland and from the adrenal and the pituitary glands assist in raising the basal metabolic level (pages 681 and 704).

The temperature-regulating mechanism of homothermic animals is inhibited during hibernation, or when curarine is injected. Under these circumstances, warm blooded animals become poikilothermic, that is, their body temperature and basal metabolism vary with the environmental temperature. Infants have a deficient temperature-regulating mechanism and cannot withstand prolonged exposure.

SPECIFIC DYNAMIC ACTION

The metabolic rate is temporarily increased above the basal level after the ingestion of food. For this reason, a caloric intake exactly equivalent to the basal metabolism would stimulate the subject to daily generation of approximately 200 more calories than are available in the food. More than the basal caloric value must, therefore, be supplied in the diet (Table 23). This stimulating effect of foods on the energy output is called the specific dynamic action. It is not due to the work performed in the digestion of foods, or to the excretion of nitrogenous waste products by the kidney. The major stimulus is provided by protein foods, and, more specifically, by certain amino acids which are the hydrolytic products of proteins. Only five of the amino acids contribute appreciably to the calorogenic effect; they are phenylalanine, glycine, alanine, tyrosine, and leucine, in order of decreasing effect. These amino acids are effective whether administered by mouth or intravenously; another amino acid, histidine, exhibits some specific dynamic action after intravenous injection. Phenylalanine and tyrosine exert less specific dynamic action in the infant than in the adult. The specific dynamic action of proteins is traceable to the intermediate metabolism of the nitrogen of the above amino acids in the liver (page 429).

Each 100 calorie portion of dietary protein gives an average specific

TABLE 23
APPROXIMATE DAILY FOOD REQUIREMENTS
IN TERMS OF CALORIES

MEN

	TOTAL FOR 70 KG. MAN	CAL. PER KG. PER DAY	CAL. PER POUND PER DAY
Basal metabolic requirement (no meals) .	1700	25	11
+ 200 Cal. (at rest with meals) ¹ . .	1900	27.5 (25 to 30)	12
+ 500 Cal. (sedentary with meals) . .	2200	30	14
+ 800 Cal. (light work with meals) ² .	2500	35	15
+ 1300 Cal. (average work with meals) ³	3000	45	20
+ 2300 Cal. (heavy work with meals) ⁴ .	4000	55	25

WOMEN

All values 15 per cent lower than those for men.

CHILDREN

AGE (YEARS)	TOTAL	CAL. PER KG. PER DAY	CAL. PER POUND PER DAY
14 to 18			
Boys	3000	45	20
Girls	2500	45	20
12 to 14	2700	45	20
10 to 12	2400	45	20
8 to 10	2100	45	20
6 to 8	1800	65	30
4 to 6	1500	65	30
2 to 4	1300	75	35
1 to 2	1000	90	40

INFANTS

AGE (MONTHS)	TOTAL	CAL. PER KG. PER DAY	CAL. PER POUND PER DAY
9 to 12	800	90	40
6 to 9	750	100	45
3 to 6	700	110	50
0 to 3	550	130	60

¹ Patients in bed.

² Professional and business men.

³ Artisans, mechanics, and tradesmen.

⁴ Laborers, farmers, and athletes.

dynamic action of 30 per cent, or 30 extra calories. The specific dynamic action of carbohydrate is only 5 per cent; that of fat 4 per cent. A plethora of non-protein foods stimulates tissues to increased oxidation, hence the mechanism of their specific dynamic action differs from that of proteins. When the energy output of an animal has already been greatly increased

above the basal level by chemical regulation at low environmental temperatures (below 5° C.), the dietary protein has no specific dynamic action. The specific dynamic action of ingested protein appears at environmental temperatures of from 59° to 68° F. (15° to 20° C.). Above 78° F. (25° C.) non-protein foods contribute to the specific dynamic action, and the regulation of body temperature becomes physical. The energy of the specific dynamic action is, therefore, useful for maintaining the body temperature in a moderately cool environment but it cannot be used for muscular work.

EFFECTS OF MUSCLE AND NERVE ACTIVITY

Approximately one-half the normal caloric output is traceable to the muscles. The markedly increased caloric requirements during muscular work are indicated in Table 23. Metabolism is temporarily increased from 25 to 50 per cent above the basal level by such slight muscular exertion as sitting or dressing, and as much as 1000 per cent by very hard work. The maximum efficiency of muscular work, in man, is approximately 25 per cent; that is, one fourth of the extra energy liberated is convertible to mechanical energy. This compares favorably with an efficiency of 10 per cent for steam engines, 20 per cent for gasoline motors, and 40 per cent for Diesel engines. The remaining three fourths of the energy evolved is converted to heat through chemical reactions and the incidental mechanical processes associated with muscular movements. It is recognized that the training of muscles slightly increases their efficiency by allowing adaptation and minimizing the incidental processes. The rate of work also affects muscular efficiency; less energy is used, per unit of distance, for walking than for running.

The respiratory quotient increases during marked muscular exertion, and carbohydrate has been shown to be the chief emergency fuel for muscles. The large amount of heat which appears as a by-product of muscular work can be used by the body to maintain its temperature during exposure to cold.

Nervous or mental efforts which do not involve simultaneous muscular activity do not cause any significant changes in total energy metabolism. This does not imply that nerve tissue has a low basal energy metabolism; the actual oxygen consumption of brain slices and glands is from 10 to 20 cu. mm. oxygen per mg. of dry weight per hour (*i.e.*, Q_{O_2} = 10 to 20). White matter is only a third as active as gray matter. Bone, resting muscle, and peripheral nerve tissue consume relatively little oxygen (Q_{O_2} = 0.5 to 5.0). A 70 kg. man uses approximately 14.67 liters of oxygen per hour, or 0.21 ml. per gram per hour. Active tissues, such as contracting muscle or secreting glands, increase their basal oxygen consumption markedly during activity. Thus, the oxygen consumption of muscle may be increased ten times and that of glands from two to four times. Section of

sympathetic or motor innervation does not immediately lower the basal metabolism of resting tissues, although denervated muscles and glands slowly undergo degenerative changes which render their metabolism subnormal.

FOOD REQUIREMENTS

From standard metabolic rates it is possible to make predictions of the approximate caloric requirements of human beings. The student should become familiar with the simple dietetic and clinical formulae of Table 23, and should make several practice calculations. These caloric requirements are normally provided by mixed diets of carbohydrate, fat, and protein. Under certain conditions, the three classes of energy-yielding foodstuffs are partly interchangeable in the diet in accordance with their caloric values. The *isodynamic law* predicts that 100 gm. of fat are the equivalent of 232 gm. of starch or of 243 gm. of protein in providing energy. In subsequent discussions of metabolism, it will be seen that each of these foods has individual virtues aside from its caloric value; hence, they are interchangeable only within certain limits. Carbohydrate is the superior foodstuff for muscular activity, while protein has special value in growth and maintenance of body weight.

The normal adult maintains a state of caloric balance; but in the growing child, or convalescing adult, a portion of the ingested food is transformed into new cellular material. Adequate diets for these subjects must contain more calories than are required for body maintenance. As shown in Table 23, the relatively high caloric requirements per unit of body weight for infants and children gradually decrease until at the eighth to tenth years they approach the lower levels of working adults. The large dietary requirements of children are due to high basal rates, great physical activity, and growth of tissue. Food deficiencies are common in children, and their diets require special attention. When insufficient calories are provided in any diet, the tissues of the subject are called on to supply the extra calories with resultant emaciation and loss of body weight. Excess caloric intake leads to obesity. (See page 249 for a detailed discussion of obesity.)

The minimal daily food allowance for the human adult, as recommended by the National Research Council, is: one pint of milk, two servings of potatoes, two servings of fruit including either a citrus fruit or tomato, two servings of vegetables including either a leafy green or yellow vegetable, one egg, one serving of meat (or of fish or poultry), one whole grain cereal dish, and, at each meal, either whole grain or enriched bread with butter or fortified oleomargarine. The daily requirement of 3000 calories for the adult male doing average work can be provided at moderate cost by using 450 gm. of carbohydrate, 80 gm. of fat, and 100 gm. of protein. Dietary protein of animal origin should be maintained at not much less than one half the total protein intake. The average distribution

TABLE 24
CALORIC VALUE OF AVERAGE SERVING OF
SOME COMMON FOODS

FOOD	WEIGHT IN GRAMS	SIZE OF PORTION	TOTAL CALORIES
Apple, fresh, raw	130	1, 2½" diam.	80
Apple pie	135	½ of 9" pie	300
Bacon, breakfast	20	4 strips, 7" long	115
Banana	125	1, 7" × 1½"	120
Beans, baked, canned	250	1 cup	230
Beans, string, canned	130	¾ cup	25
Beef, round (broiled)	115	½ pound	175
Beets, cooked	100	½ cup	35
Bread, corn	100	4½" square	275
Bread, white or rye	25	1 slice	65
Butter	10	1" × 1" × ½" cube	80
Cabbage, raw	85	½ cup	20
Candy, chocolate cream	25	2 bars	100
Cantaloupe	100	½, 5" diam.	25
Carrots, cooked	150	1 cup	50
Celery, raw	40	2, 7" stalks	7
Cheese, cottage	55	½ cup	65
Cheese, full cream	30	2" × 1" × ½" cube	130
Chicken, broiler	115	½ pound	150
Cocoa, beverage (½ milk)	154	¾ cup	100
Corn, canned	115	½ cup	100
Corn flakes	30	1 cup	115
Crackers, soda	10	3, 2" square	45
Cream (20 per cent)	15	1 tablespoon	30
Doughnuts	45	1, 3" diam.	200
Egg, whole	50	1 average	75
Egg, white	35	1 average	20
Egg, yolk	15	1 average	55
Fish:			
Halibut, steak	230	½ pound	290
Salmon, canned, sockeye	100	½ cup	190
Trout, brook, cooked	115	½ pound	130
Tuna in oil	90	½ cup	260
Oysters, "blue point"	100	6	50
French dressing	11	2 tablespoons	60
Ginger snaps	30	8, 1½" diam.	155
Grapes, Malaga	100	15	75
Grape juice (not sweetened)	120	½ cup	75
Grapefruit	100	½, 4" diam.	50

TABLE 24 (Cont.)
CALORIC VALUE OF AVERAGE SERVING OF
SOME COMMON FOODS

FOOD	WEIGHT IN GRAMS	SIZE OF PORTION	TOTAL CALORIES
Ham, smoked, lean . . .	230	$\frac{1}{2}$ pound	630
Honey, white clover . . .	100	$\frac{1}{2}$ cup	335
Ice cream, vanilla . . .	112	$\frac{1}{2}$ cup	200
Ice or sherbet	98	$\frac{1}{2}$ cup	180
Lamb chop, broiled . . .	115	$\frac{1}{2}$ pound	225
Lettuce	50	2 large leaves	6
Liver, beef	230	$\frac{1}{2}$ pound	310
Macaroni, boiled	240	1 cup	220
Mayonnaise, com.	20	1 tablespoon	150
Milk, whole	240	1 cup	170
Milk, evaporated	15	1 tablespoon	20
Milk, condensed	25	1 tablespoon	85
Oats, rolled, cooked . . .	100	$\frac{1}{2}$ cup (scant)	90
Olives, green	25	5	75
Olives, ripe, canned . . .	23	5	60
Oranges :	100	1 small	55
Orange juice	120	$\frac{1}{2}$ cup	65
Peaches	150	1 medium	60
Peanuts	60	$\frac{1}{2}$ cup, or 30	340
Peanut butter	15	1 tablespoon	95
Pears, Bartlett	150	1, 3" long	60
Peas, green, cooked . . .	70	$\frac{1}{2}$ cup	85
Pecans :	25	6 (whole)	190
Pineapple	150	1 cup, diced	65
Pork chops, loin, lean . .	230	$\frac{1}{2}$ pound	600
Potato, white, boiled . . .	150	1 medium	145
Potato, sweet, baked . . .	100	$\frac{1}{2}$ large	150
Prunes, dried, cooked . .	100	4 medium	125
Rice, boiled	100	$\frac{1}{2}$ cup	110
Spinach, cooked	100	$\frac{1}{2}$ cup	13
Strawberries	100	$\frac{1}{2}$ cup	35
Sugar, cane, granulated . .	13	1 tablespoon	50
Syrup, maple	25	1 tablespoon	75
Syrup, corn	20	1 tablespoon	60
Tomato, fresh, ripe . . .	125	1 small	25
Turnips, white	120	$\frac{1}{2}$ cup, diced	40
Veal, leg, lean	230	$\frac{1}{2}$ pound	290
Wheat, cream of, cooked . .	70	$\frac{1}{2}$ cup	115
Wheat, shredded	30	1	120

TABLE 25
CALORIC VALUE OF SOME INFANT FOODS

FOOD	WEIGHT IN GRAMS	SIZE OF PORTION	TOTAL CALORIES
Corn syrup	40	1 ounce	100
Milk, breast	30	1 ounce	20
Milk, cow's (whole)	30	1 ounce	20
Milk, evaporated	28	1 ounce	45
Buttermilk	30	1 ounce	12
Milk powder, whole	8	1 tablespoon	40
Protein-milk, powdered	8	1 tablespoon	35
Mellin's food	15	2 tablespoons	55
Nestle's food	8	1 tablespoon	40
Pablum	5	2 tablespoons	20
Cod liver oil	3.5	1 teaspoon	32
Haliver oil	2.4	$\frac{1}{2}$ teaspoon	20
Orange juice	30	1 ounce	15

of calories should be as follows: 25 per cent from cereals, beans, and potatoes; 25 per cent from fats, sugars, and accessories; 20 per cent from dairy products; 20 per cent from vegetables and fruits; and 10 per cent from meat, fish, and eggs. The caloric values of some common foods are given in Tables 24 and 25.

PATHOLOGY OF BASAL METABOLISM

"The progress of science always depends upon our questioning the plausible, the respectfully accepted, and the seemingly self-evident." — MORRIS R. COHEN

THYROID DISEASES

Endocrine diseases, especially those affecting the thyroid gland, are responsible for many clinical abnormalities of the basal metabolic rate. The latter may increase to as much as plus 100 per cent, paralleling the degree of *hyperthyroidism*. Hyperthyroid patients expend more energy in doing muscular work, which they perform with increased nervousness and unnecessary activity. The pulse rate is accelerated, and the degree of tachycardia parallels the increase in the basal metabolic rate. Because of the increased caloric demands there is a marked tendency to lose weight. It is, therefore, important to reduce the energy output by rest and to increase the dietary caloric provision. The R.Q. of fasting hyperthyroid patients is often low, because of increased utilization and rapid depletion of the carbohydrate supply, and increased formation of glucose from

proteins. Similar metabolic changes can be produced in human subjects by the administration of thyroid, or of thyroxine. The injection of 1 mg. of thyroxine causes a 2.8 per cent increase in the basal metabolic rate of hypothyroid patients, and about four times that amount in normal persons. The basal metabolic rate is dependent on the balance in tissues between the quantities of thyroid secretory product (or thyroxine) and certain antithyroid substances, such as paraxanthine.

The metabolic abnormalities and symptoms of hyperthyroid disease can often be alleviated by surgical removal of a proper amount of the abnormally active thyroid tissue. The symptoms can be relieved temporarily by the administration of iodine or iodide, which is also an accepted treatment in the preparation of patients for thyroid surgery. The use of iodides for periods exceeding a few weeks often causes an undesired secondary metabolic increase and aggravation of symptoms. Similar administration of iodine has no alleviating effect on otherwise normal persons whose metabolism has previously been elevated by thyroid medication. Thiouracil depresses synthesis of thyroid hormone and relieves the symptoms of hyperthyroidism, including the elevated basal metabolic rate. Use of this drug is an established and successful therapy for thyrotoxicosis and its preoperative management (pages 634 and 709).

The basal metabolic rate is an important diagnostic and prognostic aid in thyroid disease, because it gives a picture of the state of activity of this endocrine gland. It serves to differentiate benign adenomas and colloid goiters from toxic cases; and it also aids in discriminating between psychoneurotic conditions and true hyperthyroidism. The basal metabolic rate is useful in determining the most opportune time for operating, *viz.*, when the basal rate is falling. The pulse rate serves as an additional prognostic sign, since it often parallels the basal metabolic rate.

In the hypothyroid conditions, *cretinism* and *myxedema*, the metabolic changes are opposite to those of hyperthyroidism, and the basal metabolism may fall to minus 40 per cent in complete suppression of thyroid function. These patients gain weight and show bradycardia, in conjunction with diminished nervous and mental activities. Part of the weight increase is due to obesity, or fat deposition, and part is due to an accumulation of fluid and abnormal mucoid protein in the subcutaneous tissues (*myxedema*). The decreased metabolism and accompanying symptoms can be alleviated by administering thyroid by mouth, if treatment is not too long delayed. Basal metabolic determinations aid in differentiating myxedema from other forms of obesity and from mental disorders; they also serve as criteria for establishing the dose of thyroid.

It is now recognized that basal metabolic rates cannot be followed blindly in the diagnosis of thyroid dysfunctions. At times, the treatment of myxedematous patients effects clinical recovery but does not increase the metabolic rate. Accessory criteria, such as body weight, heart rate, nervous response, and diarrhea, are used in determining the tolerance of

patients to thyroid. Determinations of the basal metabolic rate may be misleading during remissions of hyperthyroidism, or in abnormal varieties of the disorder. Certain types of hyperthyroidism do not exhibit high basal rates, while occasional cardiac conditions do (Table 26). Despite these exceptions, the great majority of marked alterations in basal metabolic rate are due to functional disturbances of the thyroid gland.

OTHER ENDOCRINE DISORDERS

It is well known that the adrenal medullary hormone, adrenaline, increases the metabolic rate of tissues. Adrenaline exerts a rapid temporary stimulus, whereas the action of thyroxine is slow and cumulative. Approxi-

TABLE 26
CONDITIONS ACCOMPANIED BY ABNORMAL
BASAL METABOLIC RATES

INCREASES ABOVE PLUS 10 PER CENT		DECREASES BELOW MINUS 10 PER CENT	
Condition	Per Cent of Cases	Condition	Per Cent of Cases
Hyperthyroidism . . .	99	Starvation (prolonged) .	100
Hodgkin's disease . . .	Majority	Narcosis, anesthesia . .	100
Leukemia	90	Myxedema	99
Fever ¹	Majority	Cretinism	80
Polycythemia	50	Chronic nephrosis . . .	Majority
Sprue	50	Malnutrition	Majority
Acromegaly, gigantism .	40	Traumatic shock	Majority
Malignancy	40	Hypopituitarism	50
Encephalitis	20	Diabetes mellitus	30
Pregnancy	20	Addison's disease	15
Hypertension	20	Epilepsy	15
Paget's disease	15		
Heart disease	10		
Diabetes insipidus . . .	At times		

¹ The B.M.R. is raised approximately 7 per cent for each Fahrenheit degree of fever.

mately 60 per cent of the basal metabolism is maintained in the absence of both thyroxine and adrenaline. It is through adrenaline secretion that emotional states temporarily raise the basal metabolic rate, pulse rate, and blood pressure. Adrenaline increases the respiratory quotient, partly by facilitating the entrance of lactic acid from the tissues into the blood stream. Basal metabolism is somewhat low in hypoadrenal conditions, including Addison's disease; in the latter, there is a deficiency of the adrenal cortical hormones.

The pars intermedia of the pituitary gland contains a substance which stimulates metabolism, and the anterior lobe of the gland has intimate functional associations with the thyroid and adrenal glands (pages 681, 688 and 689). High metabolic rates are sometimes found in diabetes insipidus and in Cushing's syndrome, and also in the early stages of acromegaly before the hypopituitary phase appears. When the polyuria of diabetes insipidus is reduced by the administration of pituitrin, the basal metabolic rate returns to normal. Low values have been reported in hypopituitarism and in Simmonds' disease. The low basal rates for women (Table 23, page 115) indicate that the sex glands exert indirect influences on metabolism. Obesity is closely related to the problem of excess caloric intake. Obese patients do not have abnormal basal rates unless endocrine complications are present.

PREGNANCY

During the last three months of pregnancy, the mother's basal metabolism increases and becomes proportional to the combined areas of the mother and fetus. This is further proof that surface area correlations are not directly related to cooling. Increased thyroid activity may, at times, raise the metabolism of pregnant women. The mother's metabolism returns to normal shortly after parturition. Occasionally, the metabolism of women is increased during lactation and premenstrually; it is lowered during menstruation.

FEVER

In the initial stage of a febrile condition the elimination of heat fails to keep pace with the increased metabolism. Later, both the production and elimination of heat are accelerated; and in chronic wasting infections there is a considerable toxic metabolism of body proteins. Cellular oxidation is accelerated whenever the body temperature is elevated, because the speed of chemical reactions is increased by rising temperature. It is, therefore, routine to ascertain the body temperature prior to determination of the basal metabolic rate.

STARVATION

In severe malnutrition, or in prolonged starvation, the basal metabolic rate may fall from 15 to 30 per cent, partly because of an inadequate supply of protein. When adequate diets are administered to these patients, the basal rates return to normal. Ketone acids accumulate in the body during starvation, and these acids liberate carbon dioxide from the alkali reserve thus causing false high respiratory quotients. It is obvious that the exposure of starving patients to cold will cause an increased loss of body weight because of the increased caloric demand.

DIABETES MELLITUS

The totally diabetic animal suffers a marked reduction in the fuel value of all classes of ingested foods, inasmuch as glucose is formed from these foods and is excreted in the urine. The percentage caloric loss is 100 for carbohydrate food, 22 for fat, and 62 for protein, or approximately one half the normal fuel value of a mixed diet. The respiratory quotient is also lowered (Table 21, page 109).

The basal rates of diabetic patients are found, by actual measurement, to be maintained near normal, and sometimes they are even high owing to ketosis or infections. In mild cases, no change is expected either in the basal metabolic rate or in the respiratory quotient. Human beings seldom become totally diabetic; energy losses do occur, but they are less than in totally diabetic animals. Since the basal metabolic rate is maintained at the normal level, wasting of tissues may be expected in severe diabetes as the tissues are consumed to make energy replacements. The basal metabolism of diabetics may be lowered by chronic malnutrition and by wasting of tissues.

MISCELLANEOUS

Miscellaneous conditions in which the basal metabolism is altered are recorded in Table 26. Cardiac patients accumulate a greater oxygen debt than normal persons do, and they have increased metabolic rates during decompensation. Dyspnea and impaired efficiency of the heart are chiefly responsible for these effects. Similar conditions are encountered in patients with chronic respiratory disease.

Nephrotic patients may have a basal metabolism of minus 20 per cent; and, frequently, they can tolerate larger doses of thyroid than can be administered safely to normal persons. In nephritis, there is little change in the basal metabolism. The lowered basal rate in nephrotic patients does not necessarily indicate a state of hypothyroidism, but may be associated with the rapid accumulation of fluid in the tissues.

The metabolism of premature infants is lower than that of full term infants. Children show considerably greater normal variations in basal metabolic rates than do adults. These are easily misinterpreted as being pathological, but metabolic determinations are seldom necessary in children.

Alcohol does not affect the basal metabolic rate, while caffeine, dinitrophenol, and atropine cause increased rates. Dinitrophenol has very marked calorogenic effects, but it is also toxic, and, hence, cannot replace thyroid in alleviating the symptoms of hypothyroidism. Morphine causes the basal metabolic rate to fall; it provokes retention of carbon dioxide and false low respiratory quotients. Iodine and iodide, despite their effects in hyperthyroid patients, have no effect on the basal metabolism of normal persons.

BIBLIOGRAPHY

COLLOIDS

General Chemistry

- BULL, H. B. *Physical Biochemistry*. New York, Wiley, 1943.
BURTON, E. F. *The Physical Properties of Colloidal Solutions*. Ed. 3. New York, Longmans, Green, 1938.
HAUSER, E. F. *Colloidal Phenomena*. New York, McGraw-Hill, 1939.

Surface Phenomena

- ADAM, N. K. *The Physics and Chemistry of Surfaces*. Ed. 3. New York, Oxford Univ. Press, 1941.
JUST, E. E. *Biology of the Cell Surface*. Philadelphia, Blakiston, 1939.
MANTELL, C. L. *Adsorption*. New York, McGraw-Hill, 1944.
NEURATH, H., and BULL, H. B. Surface activity of proteins. *Chem. Rev.*, 23 : 391, 1938.

Electrokinetic Phenomena; Electrolytes

- ABRAMSON, H. A., *et al.* *Electrophoresis of Proteins and the Chemistry of Cell Surfaces*. New York, Reinhold, 1942.
VOET, A. Quantitative lyotropy. *Chem. Rev.*, 20 : 169, 1937.

Osmotic Pressure

- LUCKÉ, B., and McCUTCHEON, M. The living cell as an osmotic system. *Physiol. Rev.*, 12 : 68, 1932.
SCHREINEMAKERS, F. A. H. *Lectures on Osmosis*. New York, Nordemann, 1938.

Membranes

- FERRY, J. D. Ultrafilter membranes and ultrafiltration. *Chem. Rev.*, 18 : 373, 1936.
HEDGES, E. S. *Liesegang Rings and Other Periodic Structures*. London, Chapman and Hall, 1932.

FUNCTION AND PATHOLOGY OF COLLOIDS

General

- American Society of Plant Physiologists. *The Structure of Protoplasm*. Ames, Iowa State College Press, 1942.
HÖBER, R., *et al.* *Physical Chemistry of Cells and Tissues*. Philadelphia, Blakiston, 1945.
LICHTWITZ, L., *et al.* *Medizinische Kolloidlehre*. Dresden, T. Steinkopff, 1935.
PICKENS, L. E. R. Structure of biological systems. *Biol. Rev. Cambridge Phil. Soc.*, 15 : 133, 1940.

Hemolysis; Sedimentation

- HAM, T. H., and CURTIS, F. C. Sedimentation rate of erythrocytes. *Medicine*, 17 : 447, 1938.

- NICHOLS, R. E. Critical survey of erythrocyte sedimentation. *J. Lab. & Clin. Med.*, 27 : 1317, 1942.
- PONDER, E. The Mammalian Red Cell and the Properties of Hemolytic Systems. Berlin, Gebrüder Borntraeger, 1934.

Permeability

- BROOKS, S. C., and BROOKS, M. M. The Permeability of Living Cells. Ann Arbor, Edwards, 1941.
- Cold Spring Harbor Symposia Quant. Biol. Vol. VIII. Permeability and the Nature of Cell Membranes. New Bedford, Darwin Press, 1940.
- DAVSON, H., and DANIELLI, J. F. The Permeability of Natural Membranes. London, Cambridge Univ. Press, 1943.
- FRIEDEMANN, U. Blood-brain barrier. *Physiol. Rev.*, 22 : 125, 1942.

Lymph and Tissue Fluid

- DRINKER, C. K., and YOFFEY, J. M. Lymphatics, Lymph and Lymphoid Tissue. Cambridge, Harvard Univ. Press, 1941.

Inflammation

- MENKIN, V. Dynamics of Inflammation. New York, Macmillan, 1940.
- STENN, F. Hyperergic inflammation. *Arch. Path.*, 26 : 244, 1938.

Placental Permeability

- HUGGETT, A. S. G. The nutrition of the fetus. *Physiol. Rev.*, 21 : 438, 1941.
- NEEDHAM, J. Biochemistry and Morphogenesis. London, Cambridge Univ. Press, 1942.
- NEEDHAM, J. Chemical Embryology. New York, Macmillan, 1931. (3 vol.)

Blood Clotting

(See references to vitamin K, pages 676 and 678.)

- ANDRUS, W. D., and LORD, J. W., JR., Physiology of plasma prothrombin. *Surgery*, 12 : 801, 1942.
- CHARGAFF, E. The coagulation of blood. *Adv. in Enzymol.*, 5 : 31, 1945.
- D'ALESSANDRO, A. J. Heparin: its properties and clinical uses. *Internat. Abstr. Surg.*, 74 : 62, 1942.
- KARK, R. Recent developments in hemophilia. *Clinics*, 2 : 15, 1943.
- MACFARLANE, R. C. The mechanism of hemostasis. *Quart. J. Med.*, 10 : 1, 1941.
- NYGAARD, K. K. Hemorrhagic Diseases. St. Louis, Mosby, 1941.
- QUICK, A. J. Hemorrhagic Diseases and the Physiology of Hemostasis. Springfield, Thomas, 1942.
- QUICK, A. J. The anticoagulants effective *in vivo*. *Physiol. Rev.*, 24 : 297, 1944.
- (Series of authors.) Chemical, clinical and immunological studies on products of human plasma fractionation. *J. Clin. Investigation*, 23 : 417-606, 1944.
- SILBERBERG, M. Causes and mechanism of thrombosis. *Physiol. Rev.*, 18 : 197, 1938.

- TOCANTINS, L. M. The mammalian blood platelet in health and disease. *Medicine*, 17 : 155, 1938.
- WRIGHT, I. S., and PRANDONI, A. Action of dicoumarin in man. *J. A. M. A.*, 120 : 1015, 1942.

Calculi

- CARTER, R. F., *et al.* Etiology of gallstones. *Arch. Surg.*, 39 : 691, 1939.
- HIGGINS, C. C. Renal Lithiasis. Springfield, Thomas, 1943.
- WEISS, S. Gallbladder and Bile Ducts. Chicago, Year Book Publishers, 1944.

Colloid Chemistry in Histology

- BANK, O., and JONG, H. G. B. DE. Physicochemical basis of metachromatic staining reactions. *Protoplasma*, 32 : 489, 1939.
- GERSH, I. Recent developments in histochemistry. *Physiol. Rev.*, 21 : 242, 1941. (Series of authors.) Frontiers in Cytochemistry. *Biol. Symposia*, Vol. 10. Lancaster, Jacques Cattell Press, 1943.
- ZEIGER, K. Physikochemische Grundlagen der histologischen Methodik. Ann Arbor, Edwards, 1944.

ENZYMES

General Chemistry

- NORD, F. F., and WEIDENHAGEN, R. Handbuch der Enzymologie. Leipzig, Akademische Verlagsgesellschaft, 1940.
- NORTHROP, J. H. Crystalline Enzymes. New York, Columbia Univ. Press, 1939.
- SUMNER, J. B., and SOMERS, G. F. Chemistry and Methods of Enzymes. New York, Academic Press, 1943. (Methods.)
- TAUBER, H. Enzyme Chemistry. New York, Wiley, 1937. (Methods.)

Individual Hydrolytic Enzymes

- BALDWIN, E. Arginase. *Biol. Rev. Cambridge Phil. Soc.*, 11 : 181, 1936.
- BELFANTI, S., *et al.* Lecithinase. *Ergebn. Enzymforsch.*, 5 : 213, 1936.
- BERGMANN, M. A. Classification of proteolytic enzymes. *Adv. in Enzymol.*, 2 : 49, 1942.
- BERGMANN, M., and FRUTON, J. S. The specificity of proteinases. *Adv. in Enzymol.*, 1 : 63, 1941.
- BREDERECK, H. Nucleases. *Ergebn. Enzymforsch.*, 7 : 105, 1938.
- FOLLEY, S. J., and KAY, H. D. Phosphatases. *Ergebn. Enzymforsch.*, 5 : 159, 1936.
- FROMAGEOT, C. Sulfatases. *Ergebn. Enzymforsch.*, 7 : 50, 1938.
- GLICK, D. The nature and significance of choline esterase. *Biol. Symposia*, 5 : 213, 1941.
- JOHNSON, M. J., and BERGER, J. The enzymatic properties of peptidases. *Adv. in Enzymol.*, 2 : 69, 1942.
- KERTESZ, Z. I. Pectic enzymes. *Ergebn. Enzymforsch.*, 5 : 233, 1936.
- MASCHMANN, E. Bacterial proteinases. *Ergebn. Enzymforsch.*, 9 : 155, 1943.
- NELSON, J. M. Invertase. *Chem. Rev.*, 12 : 1, 1933.
- PIGMAN, W. W. The glycosidases. *Adv. in Enzymol.*, 4 : 41, 1944.

General Functions

- AMMON, R., and CHYTREK, E. The significance of enzymes in clinical diagnosis. *Ergebn. Enzymforsch.*, 8 : 91, 1939.
- DUBOS, R. J. The adaptive production of enzymes by bacteria. *Bacteriol. Rev.*, 4 : 1, 1940.
- GREENSTEIN, J. P. Recent progress in tumor enzymology. *Adv. in Enzymol.*, 3 : 315, 1943.
- LINDERSTROM-LANG, K. Distribution of enzymes in tissues and cells. *Harvey Lect.*, 34 : 214, 1938-39.
- NORTHROP, J. H. The formation of enzymes. *Physiol. Rev.*, 17 : 144, 1937.

OXIDATION

General

- BALL, E. G. Energy relation of the oxidative enzymes. *Ann. New York Acad. Sc.*, 45 : 363, 1944.
- ELLIOTT, K. A. C. Intermediary metabolites and respiratory catalysis. *Physiol. Rev.*, 21 : 267, 1941.
- ELVEJEHM, C. A., and WILSON, P. W. Respiratory Enzymes. Minneapolis, Burgess, 1939.
- GREEN, D. E. Mechanisms of Biological Oxidation. New York, Macmillan, 1941.
- OPPENHEIMER, C., *et al.* Biological Oxidation. New York, Nordemann, 1939.
- POTTER, V. R. Biological energy transformations and the cancer problem. *Adv. in Enzymol.*, 4 : 201, 1944.
- (Series of authors.) A Symposium on Respiratory Enzymes. Madison, Univ. of Wisconsin Press, 1942.

Oxidation-Reduction Potentials

- HEWITT, L. F. Oxidation-Reduction Potentials in Bacteriology and Biochemistry. Ed. 4. London, P. S. King and Son, 1935.
- KOLLATH, W., and STADLER, P. Reduction-oxidation potentials and metabolism. *Ergebn. Physiol.*, 41 : 806, 1939.

Dehydrogenases

- THUNBERG, T. Dehydrogenases. *Ergebn. Enzymforsch.*, 7 : 163, 1938.

Oxidases; Accessory Enzymes

- AGNER, K. Verdoperoxidase. *Adv. in Enzymol.*, 3 : 137, 1943.
- BLASCHKO, H. The amino acid decarboxylases of mammalian tissue. *Adv. in Enzymol.*, 5 : 67, 1945.
- DIXON, M. Aldehyde mutase. *Ergebn. Enzymforsch.*, 8 : 217, 1939.
- EDLBACHER, S. Histidase and urocanase. *Ergebn. Enzymforsch.*, 9 : 131, 1943.
- HARVEY, E. N. Luciferase. *Ergebn. Enzymforsch.*, 4 : 365, 1935.
- MÜLLER, D. Glucose oxidase. *Ergebn. Enzymforsch.*, 5 : 259, 1936.
- NELSON, J. M., and DAWSON, C. R. Tyrosinase. *Adv. in Enzymol.*, 4 : 99, 1944.

- POLONOVSKI, M., and JAYLE, M. Animal peroxidases. *Bull. Soc. chim. biol.*, 21 : 66, 1939.
- SUMNER, J. B. The chemical nature of catalase. *Adv. in Enzymol.*, 1 : 163, 1941.
- SUTTER, H. Polyphenoloxidase. *Ergebn. Enzymforsch.*, 5 : 273, 1936.
- TAUBER, H. Ascorbic acid oxidase. *Ergebn. Enzymforsch.*, 7 : 317, 1938.
- ZELLER, E. A. Diamine oxidase. *Adv. in Enzymol.*, 2 : 93, 1942.

Carriers

- POTTER, V. R. The mechanism of hydrogen transport in animal tissues. *Medicine*, 19 : 441, 1940.
- SCHLENK, F. Enzymatic reactions involving nicotinamide and its related compounds. *Adv. in Enzymol.*, 5 : 207, 1945.
- SHIBATA, K. Cytochrome and cell respiration. *Ergebn. Enzymforsch.*, 4 : 348, 1935.
- THEORELL, H. The yellow enzyme. *Ergebn. Enzymforsch.*, 6 : 111, 1937.

ENERGY METABOLISM

Basal Metabolism

- DU BOIS, E. F. Basal Metabolism in Health and Disease. Ed. 3. Philadelphia, Lea and Febiger, 1936.

Effects of Temperature

- THAUER, R. The mechanism of temperature regulation. *Ergebn. Physiol.*, 41 : 607, 1939.

Specific Dynamic Action

- BORSOOK, H. The specific dynamic action of proteins. *Biol. Rev. Cambridge Phil. Soc.*, 11 : 147, 1936.
- WILHELMJ, C. M. The specific dynamic action of food. *Physiol. Rev.*, 15 : 202, 1935.

Tissue Respiration

- CLARK, A. J., *et al.* The Metabolism of the Frog's Heart. Edinburgh, Oliver and Boyd, 1938.
- QUASTEL, J. H. Respiration in the central nervous system. *Physiol. Rev.*, 19 : 135, 1939.
- SCHNEIDER, E. C. Physiology of Muscular Activity. Ed. 2. Philadelphia, Saunders, 1939.
- UMBREIT, W. W., *et al.* Manometric Techniques and Related Methods for the Study of Tissue Metabolism. Minneapolis, Burgess, 1944.

Nutritional Requirements

(See references to Dietary Treatment, page 173.)

- American Medical Association. Handbook of Nutrition. Chicago, 1943.
- COOPER, L. F., *et al.* Nutrition in Health and Disease. Ed. 9. Philadelphia, Lippincott, 1943.

- DANN, W. J., and DARBY, W. J. The appraisal of nutritional status in humans. *Physiol. Rev.*, 25 : 326, 1945.
- HAWLEY, E. E., and MAURER-MAST, E. E. The Fundamentals of Nutrition. Springfield, Thomas, 1943.
- JOLLIFFE, N., and MOST, R. N. The appraisal of nutritional states. *Vitamins and Hormones*, 1 : 120, 1943.
- MACY, I. G. Nutrition and Chemical Growth in Childhood. Springfield, Thomas, 1942.
- MARRIOTT, W. M., and JEANS, P. C. Infant Nutrition. Ed. 3. St. Louis, Mosby, 1941.
- SHERMAN, H. C., and LANGFORD, C. S. Essentials of Nutrition. Ed. 2. New York, Macmillan, 1943.

PATHOLOGY OF BASAL METABOLISM

- DU BOIS, E. F. Basal Metabolism in Health and Disease. Ed. 3. Philadelphia, Lea and Febiger, 1936.

CHAPTER III

DIGESTION



FUNCTION

"The physician must adjust himself to the peculiarities of the individual; this adjustment is an art and involves a subtle sense for many imponderable factors." — MORRIS R. COHEN

INTRODUCTION

The digestion of individual foodstuffs will be discussed in subsequent chapters. Here, we shall consider the general functions of the gastro-intestinal tract and its contributory glands. Although these glands are largely secretory, they are also partly excretory in nature. They include the salivary, gastric, pancreatic, and intestinal glands, and the liver. Their chief function is to supply the hydrolytic enzymes necessary for digestion; but the liver and pancreas also perform important metabolic functions and the pancreas provides certain internal secretions. All foods, except water, salts, vitamins, sterols, and the simpler sugars, require preliminary enzymatic hydrolysis prior to absorption in the small intestine. The necessity for the digestion of the high molecular colloids, which form the bulk of food solids, is explained under the metabolism of proteins. Tissues rebuild specific protoplasmic constituents from the products of digestion; hence, in addition to making foods soluble and diffusible, digestion renders them compatible with normal tissue metabolism.

Micro-organisms play a major role in the digestive processes of herbivorous animals; in man, they are responsible for the digestion of a portion of food cellulose and pentosans. They also produce putrefactive substances in the normal large intestine, and in other portions of the gastro-intestinal tract during disease. Man and the carnivorous animals, which consume easily digestible foodstuffs, have anatomically simple stomachs, whereas ruminants have multicamerate stomachs in which micro-organisms are harbored for the digestion of cellulose and fiber.

Most of the digestive glands are provided with both nervous and chemical (hormonal) stimulatory mechanisms. The secretory response to nervous excitation is rapid but temporary, whereas hormonal stimulation causes more prolonged secretion. Some of the important characteristics of gastro-intestinal secretions are recorded in Table 27. The pH

TABLE 27

SECRETIONS OF THE ADULT HUMAN DIGESTIVE TRACT

SECRETION	STIMULATED BY	INHIBITED BY	APPROX. VOLUME ml./day	NORMAL pH RANGE	CHARACTERISTIC CONSTITUENTS
Saliva	Acetylcholine ¹ Physostigmine ¹ Pilocarpine ¹	Atropine	1300	6.3-7.0	Ptyalin
Mucus	Pilocarpine ¹			7.0-7.5	Lysozymes, sulfomucin, mucin
Gastric juice	Gastrin (histamine) ² Digested food in the intestine Nicotine ¹ Pilocarpine ^{1,3}	Enterogastrone Atropine Dilute hydrochloric acid Excess alkali	2500	Adult 0.9-1.6 Infant 5.0 ⁴	Gastric lipase, hydrochloric acid, intrinsic factor, pepsin, rennin
Pancreatic juice	Secretin Pancreozymin Pilocarpine ¹ Physostigmine ¹	Atropine ¹	700	7.5-8.0	Amylopsin, carboxypeptidase, chymotrypsinogen, lecithinase, maltase, nucleogelase, phosphatase, prokinase, protaminase, purine nucleosidase, steapsin, trypsinogen
Bile (hepatic)	Bile salts Secretin	Sugars	900	7.5-8.5	Bile salts, phosphatase
Bile (bladder)	Cholecystokinin ⁵ Pilocarpine ⁵ Acetylcholine ⁵ Physostigmine ⁵	Bile salts	400	5.5-7.0	Bile salts, phosphatase
Succus entericus	Secretin Enterocrinin Pilocarpine ¹	Atropine ¹	3000	7.1-7.6 (Colonic juice 8.0)	Amino-peptidase, ⁶ dipeptidase, ⁶ enterokinase, intestinal lipase, lactase, maltase, nuclease, phosphatase, sucrase
Gastric mucosa					Gastrin, mucinogen, pepsinogen, prorennin
Intestinal mucosa	Villikin ⁷ Physostigmine ⁵ Acetylcholine ⁵				Amino-peptidase, amylase, cholecystokinin, dipeptidase, enterocrinin, enterogastrone, enterokinase, esterase, lactase, lipase, lysozymes, maltase, mucinogen, nuclease, pancreozymin, phosphatase, prolidase, prolinase, prosecretin, purine nucleosidase, sucrase, villikin

pH ranges of gastro-intestinal contents: Adult gastric contents, 1.3 to 2.5; infant gastric contents, 5.0 to 6.0; duodenal contents, 6.0 to 7.0; adult feces, 6.9 to 7.3; infant feces, 5.0 to 7.0

¹ Affects nervous secretion.

⁵ Stimulates motility only.

² Parietal cell secretion.

⁶ The mixture of these two enzymes was formerly called erepsin.

³ Chief cell secretion.

⁷ Stimulates motility of villi.

⁴ First seven months.

optima for digestive enzymes may be found in Table 19, page 87; nomenclature and substrates of digestive enzymes are given in Table 17, page 82.

SALIVA

Of the three salivary secretions, parotid saliva is more serous and contains less organic material than either submaxillary or sublingual saliva. The latter is a distinctly mucous secretion, while submaxillary saliva is intermediate and variable. These differences are related to the proportions of serous and mucous secretory cells in the respective glands. Mixed saliva contains approximately 600 mg. per cent of solids, including 200 mg. per cent of inorganic salts, and approximately 300 mg. per cent of plasma proteins and sulfomucin (mucin). The calcium and inorganic phosphorus concentrations are approximately 6 and 17 mg. per cent, respectively. Saliva also contains an α -amylase, *ptyalin*, which hydrolyzes starch and glycogen to dextrins and maltose at an optimum pH of 6.6. This enzyme is absent from the saliva of the cat, dog, goat, and sheep. Ptyalin is activated by the chlorides present in saliva. The digestive action of ptyalin continues for a short interval in the upper food layers of the stomach contents. In the more acid portions of stomach contents, ptyalin is inactive, since it is rapidly destroyed at pH 4 or less. Cooking of starch foods increases their palatability by rupturing the envelopes surrounding the starch grains and exposing the starch to ptyalin action. Salivary digestion of foods is slight and is limited to the polysaccharides (starch, glycogen, and dextrins).

Secretion of Saliva

A specific excitatory hormone for the salivary glands is unknown; however, the hormone of parasympathetic nerve endings, *acetylcholine*, acts as a salivary secretagogue. Plasma and tissues contain an enzyme, *choline esterase*, which destroys the acetylcholine liberated at parasympathetic nerve endings. The activity of choline esterase is inhibited by prostigmine and physostigmine; administration of these substances or pilocarpine causes increased salivation because of prolongation of the effects of parasympathetic stimuli. Atropine counteracts the action of acetylcholine, inhibits salivation, and produces dryness of the mouth and throat. The absence of hormonal excitation of the salivary glands may possibly be correlated with the brief retention of food in the oral cavity.

The nerves supplying the salivary glands are easily excited reflexly by stimulation of afferent nerves of the oral, esophageal, or gastric mucosa. Hence, the glands respond to food intake, to psychic activity, and to irritation of the stomach or esophagus. Stimulation of the taste organs causes an unconditioned reflex, while sounds, cutaneous sensations, and the sight or smell of food produce conditioned reflexes. The unconditioned reflex follows within two or three seconds after the introduction of food into the mouth. The amount and quality of saliva vary with the

nature of the food, and with its chemical stimulation of the taste buds. Inert substances provoke salivation by physical stimulation of the common sensibility fibers in the mouth. The secretion of saliva is related to the sensation of thirst. Water deficiency causes stimulation of the sensory nerve endings in the pharynx.

GASTRIC JUICE AND GASTRIC ENZYMES

Gastric juice is a dilute secretion which contains hydrochloric acid, enzymes, sulfomucin (mucin), and salts. There are two important functional divisions of the stomach, namely, the fundus and the pylorus. The parietal or oxyntic cells, which secrete the hydrochloric acid, are situated in the fundic glands in close proximity to the zymogenic chief cells. The important enzymes of gastric juice are pepsin, rennin, gastric lipase, and the intrinsic factor. Sulfomucin is a glycoprotein secreted by a third type of cell, the goblet or mucus cell. Pepsinogen and mucinogen granules, which contain precursors of pepsin and sulfomucin, are histologically demonstrable in the chief cells and the goblet cells, respectively. Details of the activation of pepsinogen by hydrochloric acid and by pepsin have been given on page 80. The proteolytic enzymes disappear from gastric mucosa which has become neoplastic.

During the process of gastric digestion, *pepsin* hydrolyzes most native proteins to form the higher cleavage products: metaproteins, proteoses, and peptones. Even during prolonged digestion *in vitro*, it liberates only relatively small quantities of amino acids. Pepsin is a protein with an optimum pH of approximately 2.0. As with other proteolytic enzymes, its pH optimum varies somewhat with the nature of the substrate, being 1.8 with casein and 2.2 with gelatin. Pepsin activity is abolished above pH 5.0, and the enzyme is rapidly destroyed in solutions more alkaline than pH 8.0. Above pH 5.5, pepsin is digested by the pancreatic enzyme, trypsin; and the latter is digested by pepsin at a lower pH. It is evident that the normal digestive action of pepsin is limited to the gastric cavity. Certain parasites contain antipepsin which favors the survival of these organisms in the gastro-intestinal tract.

The specific casein-digesting or milk-curdling enzyme, *rennin*, is found in largest quantities in the gastric juice of infants and young animals. Rennin is an incoagulable, diffusible protease with an optimum pH of 3.5. It exists in the chief cells as a zymogen, prorennin, which is activated at an acidity greater than pH 5.0.

Gastric juice contains a fat-hydrolyzing enzyme, *gastric lipase*, which differs from the more powerful lipases of the intestinal tract by acting best in an acid medium (optimum pH, 5.0). Gastric lipase is inactive at the low pH of adult gastric contents; hence, its digestive action is more important in infants and in conditions of hypoacidity. The action of this enzyme is limited to highly emulsified fats of the type found in milk, butter, and

egg yolk. It is evident from the foregoing discussion that the partial hydrolysis of proteins is the most important digestive function of the adult human stomach.

A less clearly characterized constituent of gastric juice is the *intrinsic factor*, necessary for the production of the hematopoietic liver substance. The latter causes a reticulocyte response, increased erythrocyte count, and improvement of the clinical symptoms of macrocytic anemias. The hematopoietic substance is formed from the *extrinsic factor* of beef muscle by incubation with normal gastric juice at pH 5 to 7. The enzymatic, thermolabile intrinsic factor is absent from the gastric juice of pernicious anemia patients.

Acidity of Gastric Juice

Gastric juice of the adult human, as obtained through stomach fistulae, contains approximately 0.12 ± 0.02 N hydrochloric acid, and 0.15 ± 0.03 N total acid ($\text{pH} = 1.2 \pm 0.3$). At this hydrogen ion concentration, the action of gastric enzymes is facilitated and the growth of micro-organisms is inhibited. Certain spores and parasites are resistant to a low pH, and may pass into the intestine in viable forms.

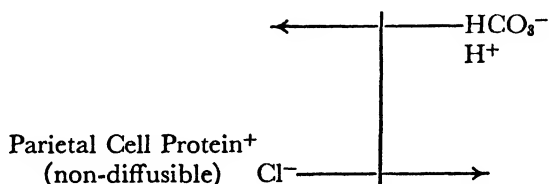
Gastric contents after an Ewald test meal (two slices of toast and a cup of tea) show the partial neutralization of gastric juice by foods, the total acid being approximately 0.06 N and the hydrochloric acid, 0.04 N. At the height of the digestion of ordinary meals the gastric contents have a pH of from 1.3 to 2.5, and contain approximately 0.08 N hydrochloric acid. It is evident that the Ewald test meal is less stimulatory to the gastric glands, and causes a greater dilution of the gastric juice than does an average meal.

Origin of Hydrochloric Acid

The mechanism of the production of hydrochloric acid by the parietal cells is not certain, but it has been proved that the acid is derived from inorganic chlorides which enter the secretory cells from the blood plasma and extracellular fluids. Gastric juice and blood are in osmotic equilibrium and have equivalent total ion concentrations. The total chloride of gastric juice is, therefore, more constant than its hydrochloric acid content. During gastric activity, the parietal cells show a remarkable increase in chloride content. Animals which have been maintained for some time on very low chloride diets no longer secrete hydrochloric acid; also, when gastric juice is lost continuously from the body, the blood chloride level falls. Microchemical studies show that very little chloride is present within the parietal cells during the resting stage. These cells contain considerable phosphate and protein which can combine with base during active gastric secretion. The rate of gastric secretion is decreased when the plasma bicarbonate is low, as in acidosis or in hyper-

ventilation. In dogs, the secretion of hydrochloric acid is diminished by thiocyanate and by high blood levels of sulfanilamide, which inhibit the activity of carbonic anhydrase. This enzyme is present in large quantities in the gastric mucosa, and it is evidently concerned in the secretory mechanism, for the rate of hydrochloric acid secretion is proportional to the rate of carbonic acid formation. The parietal cells extract chloride anion from the blood plasma, and replace it with bicarbonate ion formed from carbon dioxide by carbonic anhydrase.

A Donnan membrane equilibrium is concerned in the secretory mechanism. Proteins in the parietal cells apparently exert an influence similar to that of hemoglobin in the erythrocytes, and they induce the Donnan effect indicated in the following simplified diagram:



The bicarbonate leaves the parietal cells to enter the blood, while protons and chloride anions pass into the gastric juice. *In vitro* experiments with protein and chloride ions demonstrate that hydrochloric acid can be "secreted" in this fashion by non-living membranes. Only a minute fraction of the hydrochloric acid present in gastric juice can exist as free acid in the parietal cells, because the cytoplasmic pH of these cells is near 6.8. Formation of hydrochloric acid by isolated gastric mucosa is abolished by cyanide or iodoacetate, which interferes with carbohydrate oxidation and thus blocks the energy source for the secretory process. It should be noted that the enzyme precursors are made in one set of gastric cells and the activating hydrochloric acid in another; it is only in the tubules that the two are mixed to create an active digestive mixture. Micromanipulation experiments have shown that normal living cells are never very acid. However, damaged or ischemic tissues can no longer prevent acid accumulation, or resist the digestive action of gastric juice. These facts are important in considering the possible etiology of peptic ulcers (page 160).

Systemic Relations of Gastric Secretion

The secretion of hydrochloric acid and the consequent return of the separated cations to the venous blood have certain systemic relations. When the secretion of gastric juice begins, the plasma chloride is temporarily decreased; but it is soon replenished by chloride from other extracellular fluids and by reabsorption of chloride from the intestinal lumen.

The increased bicarbonate and decreased chloride in the plasma induce a reversed chloride shift in the erythrocytes. The cations which enter the blood and tissue fluids from the secreting parietal cells disturb the acid-base balance and cause decreased acid excretion by the kidneys. This results in an increased pH of the urine after meals, a phenomenon known as the *alkaline tide*. Accompanying the alkaline tide there are increases in the plasma bicarbonate and in the carbon dioxide of alveolar air. However, the alkaline tide is not always the result of hydrochloric acid secretion, since it has been observed in achlorhydric patients whose gastric glands are not secreting hydrochloric acid. Diuresis and an excess of base in the food are important contributory causes of the alkaline tide. The secretion of pancreatic juice and the other alkaline intestinal secretions follows so closely upon the secretion of gastric juice that their effects are counter-balanced.

Gastric Secretory Phases

There are three phases of gastric secretion. The initial phase is a *cephalic*, *psychic*, or *appetite secretion* which is stimulated by the taste of food, by conditioned reflexes, or by hypnotic suggestion. This phase is short (one-half hour, or less), but it is physiologically important for the rapid initiation of gastric secretion. The cephalic phase can be demonstrated by the sham feeding of animals which have esophageal fistulae. Nicotine and pilocarpine accelerate gastric secretion through nervous mechanisms. Nicotinic acid and other vasodilator substances also increase gastric secretion.

The subsequent *chemical* and *intestinal* phases, which account for the major portion of the gastric secretion, are incited by the action of foods and their digestion products on the pyloric and duodenal mucosae. Important physiological excitants of the gastric glands are meat, water, caffeine, dilute alcohol, and the protein digestion products which are present in meat and vegetable juices. Condiments and flavoring materials have no chemical secretory effect, although they sometimes stimulate the psychic mechanism. The chemical excitation of the gastric glands continues even after section of the vagus nerves which mediate the psychic stimuli. Chemical stimulation of gastric secretion is controlled by the hormone, gastrin.

Gastrin

This hormone is normally liberated in the gastric mucosa by the action of protein digestion products. It is transported by the blood to the fundic glands, where it incites the flow of gastric juice. Gastrin can be extracted from pyloric mucosa, *in vitro*, with dilute hydrochloric acid. While not identical with histamine, it is related chemically to the latter, since it is destroyed by contact with the enzyme, histaminase. However, administration of histaminase preparations to animals does not diminish gastric secretion.

Gastrin is formed chiefly in the pyloric region of the gastric mucosa, and is stored principally in the fundus. Histamine and gastrin activate parietal cells more than zymogenic chief cells. There is some evidence that an additional hormone of the pyloric mucosa stimulates the chief cells.

Intestinal Phase of Gastric Secretion

Gastric secretion is induced when protein and fat digestion products, such as proteoses, peptones, and soaps, are placed directly into the duodenum. It is also incited by the parenteral injection of extracts of duodenal mucosa made with dilute hydrochloric acid, which may be an indirect effect of the protein digestion products on the gastrin mechanism. Atropine prevents this effect; vagal section does not.

Inhibition of Gastric Secretion

When hydrochloric acid, or any other highly dissociated acid, is introduced into the gastric cavity of a living animal it definitely inhibits gastric secretion. There is a gastric mechanism which tends to maintain an optimal acidity for the action of the digestive enzymes. Small doses of alkali are slightly stimulatory to gastric secretion; large doses depress secretion for some time following their administration. For this reason, and also because they neutralize the acidity of the secreted gastric juice, alkaline preparations are useful in the treatment of peptic ulcer. Atropine will inhibit gastric secretion; and fear, pain, and sleep tend to reduce the secretory activity.

Enterogastrone

An important hormonal inhibitor of gastric secretion, enterogastrone, is found in the duodenal mucosa and is excreted in the urine (urogastrone). Such inhibitory hormones are termed *chalones*, to distinguish them from the usual excitatory hormones. Enterogastrone decreases the volume, the acidity, and, especially, the enzyme content of gastric juice. It appears in the intestinal mucosa whenever undigested fat enters the duodenum. Injected enterogastrone extracts completely suppress the secretion of gastric juice for a number of hours and inhibit gastric motility for a shorter time. Food fat inhibits all phases of gastric secretion and also decreases motility; hence, the fat content of the diet is of therapeutic importance.

Adjustment of Acidity of Gastric Contents

Gastric acidity is controlled by the regulation of gastric secretion together with intermittent evacuation of portions of the gastric contents into the duodenum. Swallowed saliva neutralizes and dilutes gastric juice. During digestion, duodenal fluid is regurgitated into the stomach, as is evidenced by the appearance of bile pigments and trypsin in stomach

contents, especially during the late stages of gastric digestion. The surgical prevention of regurgitation has proved that it is a minor factor in the regulation of gastric acidity. The duodenal juice is only slightly alkaline, and it is regurgitated in such small quantities that its neutralizing power is negligible.

Sulfomucin and Mucin

These glycoproteins are present in saliva, gastric juice, and intestinal secretions to the extent of approximately 200 mg. per cent. They are found in the mucous secretions of the goblet cells which are stimulated by irritation of the surface epithelia. Sulfomucin is an ampholyte with appreciable neutralizing power, but the neutralization of human gastric juice by mucus is probably of minor significance. Sulfomucin, as well as its polysaccharide component, inhibits pepsin and other proteolytic enzymes. However, sufficient pepsin will digest sulfomucin in normal or hyperacid gastric mixtures; it is also digested by trypsin in the intestine. The effects of sulfomucin on bacteria are of considerable interest. When certain micro-organisms and viruses are injected subcutaneously, intraperitoneally, or intratracheally, together with sulfomucin, the latter increases their virulence, perhaps by inhibiting phagocytosis. However, in the gastro-intestinal tract, sulfomucin exerts certain important bacteriostatic effects traceable to its polysaccharide sulfuric ester component. Thus, the resistance of chickens to ascaris infection has been shown to increase with the number of goblet cells. Mucous secretions also contain low molecular bacteriolytic proteins, the *lysozymes*, which exert additional protective effects against micro-organisms. These proteins appear to be enzymes which hydrolyze bacterial polysaccharides. Lysozymes are present in largest quantities in the colonic mucosa.

Food Relations

The adjustment of gastric acidity is related to the buffering and neutralizing capacities of the food eaten, which usually varies directly with the protein content. The normal response of the stomach to food intake is the secretion of that amount of hydrochloric acid necessary to produce a pH of approximately 1.9 in the gastric contents. The titratable acidity varies with the acid-binding value of the food mixture. These relations are summarized in Table 28.

Gastric Motility

Solid or semisolid food reaches the stomach about six to seven seconds after leaving the oral cavity. Gastric movements and the digestive action of enzymes convert the food mixture to a gruel-like material, the *chyme*. The acid chyme passes intermittently through the pylorus and the stomach is emptied in approximately four hours after the usual meal (Table

28). Normal evacuation of the Ewald meal requires two hours; water and other fluids leave more quickly. The infant's stomach empties rapidly, permitting a fluid intake that exceeds the capacity of the stomach. The stomach does not empty by gravity but rather as the result of active muscular contractions. Food fat promptly inhibits gastric motility, reduces the depth of peristaltic waves, and lowers gastric tone. It does this when introduced either into the stomach or into the intestine; in the latter, it liberates enterogastrone, which inhibits gastric secretion.

TABLE 28
EFFECT OF FOODS ON GASTRIC DIGESTION

Food	APPROXIMATE EVACUATION TIME (HOURS)	Food	UNITS OF TOTAL ACID
Fat:		Protein:	
Nuts, olive oil . . .	>4	Fowl, lamb, fish, beef, pork	120-140
Ice cream (32% cream) pork, bacon, egg yolk, chicken, turkey . .	3½	Fat:	
Protein:		Nuts, ice cream . .	100-120
Beef, lamb, fish, egg white	3	Carbohydrate:	
Carbohydrate:		Fruits, pies, eggs, cakes, puddings, bread, cereal	80-100
Bread, cereal, pies, cakes	2½	Vegetables, gelatin, sugars, candies . .	70-80
Vegetables, puddings, porridge	2½		
Fruits, gelatin, sugar, candies	2½	Ewald meal	60
Ewald meal	2	Milk	50
Milk	1½		

One of the functions of the stomach is the maintenance of a proper semifluidity of the chyme. The degree of fluidity of stomach contents is an important factor in determining the evacuation time; other influences are the osmotic pressure of the gastric contents, and the degree of distention of the duodenum. Hypertonic solutions frequently give rise to nausea; such solutions are diluted before leaving the stomach. Exercise, fasting, hunger, hemorrhage, anger, anxiety, exposure to cold, and the administration of alcohol, bile salts, coffee, histamine, insulin, sodium bicarbonate, and thyroid increase gastric motility and hasten evacuation; atropine, thiamin deficit, smoking, fear, pain, sorrow, and fever decrease motility.

Hunger

This gastric sensation is related to strong peristaltic contractions of the empty stomach. Since similar sensations are experienced after removal of the human stomach, the duodenum is also concerned. Bulky, low calorie foods allay hunger and are used in the treatment of obesity. Hunger contractions are augmented by the excitants of gastric motility listed above.

Appetite

Appetite is an acquired sensation related to gastric tonicity and partly dependent on conditioned stimuli. Dilute alcohol increases the tonus of the stomach. In thiamin deficiency, the gastric muscular tone is lowered and the appetite is lost (anorexia). Similar loss of appetite occurs in a variety of diseases. Since appetite has pronounced influences on digestion, it is important that therapeutic diets be made attractive. Psychic effects are paramount in the maintenance of appetite; they can be controlled more effectively by the intelligent application of the dietician's and physician's arts than by the use of bitter tonics and kindred devices. Thiamin, vitamin B complex, and insulin are used therapeutically to restore lost appetite.

PANCREATIC JUICE AND PANCREATIC ENZYMES

The pancreas has two secretions of great physiological importance, an external digestive secretion (pancreatic juice), and an internal secretion (insulin) which regulates carbohydrate metabolism. Pancreatic juice is usually discharged through the duct of Wirsung into the ampulla of Vater, where it joins the bile and passes into the intestinal lumen about 11 cm. below the pylorus. There is at times an accessory pancreatic duct, slightly above the duct of Wirsung, which communicates with both the intestine and the ampulla. In a few individuals, the pancreatic and common bile ducts have separate orifices. Pancreatic juice is slightly alkaline (Table 27, page 132) and contains about 1.3 per cent of solids. The pancreas is the most important zymogenic organ of the gastro-intestinal tract; the chief pancreatic enzymes are trypsin, chymotrypsins, carboxypeptidase, amylapsin, and steapsin.

The powerful proteolytic enzyme, *trypsin*, has an optimum pH of approximately 8.0; the exact value varies somewhat with the nature of the protein substrate. The acinar tissue of the pancreas secretes trypsin as an inactive zymogen — trypsinogen. The latter is found in the juice obtained from pancreatic fistulae. Trypsinogen is converted into active trypsin by *enterokinase* from the intestinal mucosa. Pancreatic juice contains an inactive or zymogenic precursor of enterokinase — prokinase. Some trypsin is formed by enterokinase and this, in turn, activates the remaining trypsinogen and the chymotrypsinogens. In the intestinal

lumen, trypsin and *chymotrypsins* hydrolyze the partially digested proteins of chyme; but they can also attack most native food proteins. Trypsin activity is inhibited by heparin and by the antitrypsins found in intestinal parasites, egg white, and blood plasma. Chymotrypsins have a greater rennetic effect than trypsin. The several trypsin hydrolyze different proteins according to the constitution of the latter. Pancreatic protaminase assists in the hydrolysis of protamines. Preliminary partial digestion of proteins by the gastric juice favors rapid digestion by pancreatic enzymes. The trypsin do not completely disintegrate protein foods into the simplest chemical units but rather to intermediate proteoses, peptones, and polypeptides. The latter are then hydrolyzed to amino acids by the peptidases of pancreatic juice, succus entericus, and intestinal mucosa. The peptidase of pancreatic juice is *carboxypeptidase*. It digests peptides at the carboxyl end of the chain.

The pancreatic amylase, *amylopsin*, is more powerful than ptyalin. Amylopsin rapidly digests starch, glycogen, and dextrans. The optimum pH of amylopsin is 7.0; it is activated by chloride anions. During the first few weeks of life, the infant's pancreatic juice does not contain amylopsin; hence, starch is then quite indigestible. In fact, the infant is prepared to digest efficiently only one food, namely, milk.

The digestion of fats is accomplished primarily by the pancreatic lipase, *steapsin*. The action of steapsin is accelerated by the bile salts which are secreted by the liver and transported in bile to the intestinal lumen. Bile salts accelerate the action of lipases, chiefly by assisting in the emulsification of fats. They inhibit the action of the closely related *esterases*. Esterases hydrolyze simple esters and lower glycerides, but they act very slowly on ordinary fats. Pancreatic juice contains a *cholesterase* which hydrolyzes sterol esters. The lipase and esterase content of pancreatic juice is apparently decreased in thiamin deficiency. Pancreatic juice also contains *lecithinase*, which digests phospholipides, and *nucleogelase*, *phosphatase*, and *purine nucleosidase*, which assist in the digestion of nucleic acids.

Secretin and Pancreozymin

Secretin and pancreozymin are the hormones which excite the secretion of pancreatic juice. Secretin is produced from prosecretin of the upper intestinal mucosa; it is liberated by the action of acid chyme and is then absorbed into the blood stream. However, pancreatic secretion continues even when the chyme does not contain hydrochloric acid; under these circumstances the secretin is probably liberated by the action of the fatty acids which are produced during the digestion of fat. Bile salts aid in the absorption of secretin from the intestinal lumen. The stimulation of the pancreatic acinar cells by secretin is independent of nervous regulation and is, therefore, uninfluenced by atropine or ergotamine. Secretin also stimulates the secretion of hepatic bile and, very probably, the secretion

of *succus entericus* by the intestinal glands. The hormone has been isolated, crystallized, and found to be a basic proteose. Secretin is active when injected intravenously but not when given orally because it is digested by pepsin, trypsin, and aminopeptidase. Subcutaneous injections are also quite inactive.

Pancreozymin, another hormone found in the duodenal mucosa, causes increased secretion of pancreatic enzymes, whereas the secretin-stimulated secretion is more serous. Vitamin A is reported to have a favorable influence on pancreatic secretion.

Vagus impulses and the administration of pilocarpine excite the secretion of a small volume of pancreatic juice which has an increased enzyme content. Atropine abolishes only the nervous excitation, which mediates the relatively unimportant psychic phase of pancreatic secretion.

BILE

Bile is a yellow, brown, or occasionally green fluid secreted by the polygonal cells of the liver. The principal bile solids are the bile salts (glycocholates and taurocholates), the bile pigments (bilirubin and biliverdin), fatty acids, soaps, cholesterol, lecithin, neutral fat, fat-soluble excretory products (pigments, phenols, alkaloids, and toxins), and certain polyvalent inorganic ions (pages 153 and 571). The excretion of fat-soluble metabolites in bile is undoubtedly facilitated by the favorable action of hepatic bile salts on the passage of fat-soluble plasma substances into the liver cells. Some biliary excretory products, especially those water-soluble substances which result from detoxifying actions of the liver, are reabsorbed in the intestine and partially excreted in the urine. Biliary cholesterol, lecithin, and bile salts are normally reabsorbed from the small intestine. Of the 20 gm. of bile salts which enter the human intestine daily, about 90 per cent returns to the liver through the portal circulation. This is termed the *enterohepatic circulation*. *Phosphatase* is the only enzyme known to occur, in significant quantities, in bile.

Hepatic bile contains approximately 2 per cent of bile salts; 700 mg. per cent of inorganic substances; 450 mg. per cent of bile pigments; 100 mg. per cent each of fatty acids, neutral fat, and cholesterol; and 60 mg. per cent of phospholipides. There are greater concentrations of fatty acids and soaps in bile than anywhere else in the body. The fatty acids and cholesterol are united to the bile salts by accessory valences. Normally, enough bile salts are present to dissolve the cholesterol. Bile pigments are excretory products of hemoglobin metabolism, but the bile salts are synthetic steride secretory products of the liver which have special digestive functions. The chemistry and metabolism of bile salts and bile pigments are considered in detail on pages 198 and 245, and on pages 536 and 547, respectively.

The pH of hepatic bile is approximately 8.0, and that of gallbladder

bile is about 6.5. There are approximately 3 per cent of solids in human hepatic bile, and 15 per cent in gallbladder bile. Bile is, therefore, considerably concentrated and slightly acidified in the gallbladder.

Functions of Bile

Bile is essential for efficient digestion and absorption of fats in the intestine. This secretion does not contain lipase, but it is the source of the bile salts which are important accelerators of lipase action. Bile salts aid digestion in two ways: (1) they assist in the emulsification of food fats, and thus accelerate the action of steapsin and of intestinal lipase; and (2) they perform an even more important function in dissolving fatty acid and sterol products of digestion, thereby facilitating lipid absorption through the small intestine. After ligation of both the pancreatic and common bile ducts, the lipases of the succus entericus and the intestinal mucosa can digest as much as 80 per cent of selected dietary fats; but much of this digested fat is lost in the feces. Bile salts are necessary for normal absorption of lipides, sterols, vitamin D, vitamin K, and carotenoids. They are not necessary for the absorption of vitamin A. At the pH of gastric juice, bile salts flocculate pepsin and rennin and thus inhibit their activity. Bile is a natural laxative because it stimulates peristalsis.

Although bile is usually regarded as an attenuating or bacteriostatic mixture, it does not inhibit the growth of all micro-organisms, as evidenced by its use in certain bacteriological culture media and by the frequent infections found in the gallbladder and in the bile passages. Bile salts increase the virulence of *Esch. coli*, *V. cholerae*, *E. typhosa*, and certain other micro-organisms, perhaps by the inactivation of intestinal bacteriophages. On the other hand, they inhibit some viruses and are bactericidal and lytic for certain streptococci, staphylococci, pneumococci, and the like; lysis of pneumococci by bile salts is known as the *Neufeld phenomenon*. The most important effect of bile on the intestinal flora is an indirect one. In its absence the intestine becomes filled with the digestion products of fats, which entrap food proteins. The resulting mixture provides a favorable medium for the putrefactive activities of micro-organisms in the large intestine. Bile lyses leukocytes and erythrocytes, and it may either increase or decrease the absorption or the activity of individual bacterial toxins. With the exception of the intestinal and biliary mucosa and liver cells, bile salts exert cytotoxic effects on tissues.

In addition to its digestive functions, the liver is important as an excretory organ and it is the site of essential metabolic functions which do not have any direct relation to digestion. The metabolic functions include the formation of plasma proteins, of heparins, and of embryonic blood; the storage of glycogen, copper, iron, the hematopoietic factor, and numerous vitamins; and a variety of detoxications. These phases of liver function are discussed in subsequent metabolic sections.

Secretion of Bile

The polygonal cells of the liver continually secrete bile of high surface activity. The secretion is increased by the injection of secretin, or by the oral administration of bile salts. The conjugated bile acids are most effective. Dehydrocholic acid stimulates the formation of more dilute bile than either glycocholic or taurocholic acids. In dogs, cinchophen causes a similar hydrocholeresis. Secretin and bile salts are true biliary stimulants or *choleretics*. Substances such as magnesium sulfate and cholecystokinin cause emptying of the gallbladder and are termed *cholagogues* to distinguish them from choleretics. Calomel and salicylates were formerly regarded as choleretics, but have been shown to be without secretory activity. They have been replaced, in modern therapy, by bile salts. Secretin has much less effect on the secretion of bile than it has on pancreatic secretion. The choleretic action of secretin may be an indirect effect since it is prevented by ligation of the pancreatic vein. Bile definitely assists the absorption of secretin from the intestine. A high protein diet is stimulatory and sugars are said to be slightly inhibitory to biliary secretion, while nerve section has little influence.

The Gallbladder

The intermittent entrance of bile coincident with the influx of chyme into the human intestine is facilitated by the gallbladder and the sphincter of Oddi. The quantities of bile and pancreatic juice that reach the intestine are related to the volume of the entering chyme. Protein and fat digestion products in chyme activate the hormone mechanism which controls the gallbladder.

Bile continues to be secreted against pressures as great as 22 mm. of mercury. The cystic duct opens at a biliary pressure of approximately 4.5 mm. of mercury, allowing the gallbladder to fill to its normal capacity of about 40 ml. When the pressure reaches 7.5 mm. of mercury, the sphincter of Oddi opens and the stored bile is evacuated into the intestine. The regulatory functions of the bladder are thus desirable, although not indispensable. The horse, deer, and rat have no gallbladder. The human organ can be removed, surgically, without permanent metabolic damage. After cholecystectomy the larger bile ducts dilate to form compensatory reservoirs.

The bile salts, bile pigments, lipides, and cholesterol of hepatic bile are normally concentrated from three to ten times by retention in the gallbladder; calcium is also concentrated slightly, and the volume of the bile is reduced by one half to two thirds. Water, chlorides, bicarbonates, and smaller amounts of lecithin and fats are absorbed by the bladder mucosa. During this process, the bile, like other gastro-intestinal secretions, remains in approximate osmotic equilibrium with the blood plasma. A Donnan equilibrium is involved, inasmuch as the bile acid anions do

not easily diffuse through the normal gallbladder. The gallbladder bile accumulates more base than is present in the plasma; some of this base is evidently present as coordination compounds of bile acids. The gallbladder mucosa adds a mucous secretion to bile, while the bile ducts dilute the bile with a serous secretion. The ducts and gallbladder are, thus, somewhat antagonistic, although the gallbladder has the more pronounced effect.

Cholecystokinin

The nervous control of the gallbladder is not understood completely; but it is known that acetylcholine, physostigmine, pilocarpine, and vagus stimulation cause evacuation. Hormonal stimulation of gallbladder contraction is due to cholecystokinin. During fasting, the sphincter of Oddi is tonically constricted and the gallbladder becomes distended with bile. The entrance of chyme into the duodenum causes opening of the sphincter of Oddi and contraction of the gallbladder musculature. Ingested fats and their digestion products cause the greatest stimulation; proteins have a smaller effect. Emptying of the gallbladder occurs in about two hours after an average meal. The introduction of fruit acids, food oils, or dilute hydrochloric acid into the duodenum induces contraction of the gallbladder musculature and evacuation of bile; petroleum oil has no effect. Magnesium sulfate promotes evacuation by relaxation of the sphincter of Oddi. The presence of fat in the duodenum stimulates contraction of the denervated gallbladder. Blood taken from one animal at the height of digestion causes gallbladder contraction when injected into a fasting animal. The injection of acid extracts of the duodenum causes contraction; but fats and hydrochloric acid are not active when given intravenously. These facts indicate that cholecystokinin is a gallbladder-stimulating hormone, liberated from the duodenal mucosa, and that it is carried to the gallbladder by the blood stream. Bile salts injected intravenously cause relaxation of the gallbladder.

SUCCUS ENTERICUS AND INTESTINAL MUCOSA

Succus entericus or intestinal juice is secreted by glands in the crypts of Lieberkühn. Brunner's glands, in the duodenum, also contribute to the secretion. The pH of succus entericus is 7.1 in the duodenum, 7.6 in the ileum, and 8.0 in the colon. The enzyme content varies considerably, and the digestive action of the juice is unquestionably inferior to the action of similar enzymes which are retained within the intestinal mucosa (Table 27, page 132). Succus entericus from the upper intestine contains aminopeptidase, dipeptidase, nuclease, phosphatase, intestinal lipase, sucrase (or invertase), maltase, lactase, and enterokinase. The intestinal mucosa contains the same enzymes plus purine nucleosidase, prolidase, prolinase, and amylase. The enzyme content is low in succus entericus from the ileum, and digestive enzymes are absent from the colonic juice.

The mixture of intestinal *aminopeptidase*, *dipeptidase*, *prolinase*, and *prolidase* was formerly known as *crepsin*. These proteolytic enzymes digest protein fragments, but are unable to act on native proteins. *Aminopeptidase* hydrolyzes polypeptides at the amino end of the chain; *dipeptidase* acts only on dipeptides; and *prolidase* and *prolinase* hydrolyze two series of special peptides (page 402). The peptidases of the intestinal mucosa prevent the entrance of partially digested proteins into the circulation. Similar peptidases are present in other tissues of the body.

The disaccharide-splitting enzymes, *lactase*, *maltase*, and *sucrase*, of the intestinal mucosa are especially important. They are the only enzymes of this type in the gastro-intestinal tract, aside from small amounts of maltase in pancreatic juice and saliva.

Succus entericus contains a small quantity of *lipase* which is important for the digestion of fats that may escape the action of the pancreatic juice. In the absence of pancreatic juice, the major portion of ingested fat is hydrolyzed by intestinal lipase, even though the resulting fatty acids are not easily absorbed. This lipase is also concerned in the resynthesis of fat during absorption (page 213). pH optima of the intestinal enzymes are given in Table 19, page 87.

Secretion of Succus Entericus

The secretion of *succus entericus* is controlled by a nerve plexus within the intestinal wall, and by the hormone, *enterocrinin*, produced by the small intestine. Stimulation of the mucosa leads to the secretion of both *succus entericus* and *mucus*. *Mucus* is secreted by the goblet cells of both the small and large intestine.

PASSAGE OF FOOD THROUGH THE SMALL INTESTINE

The first 2 inches of the small intestine, the duodenal cap, receive the acid chyme evacuated from the stomach. The pH of duodenal contents varies from 6 to 7, according to the quantity and acidity of the evacuated chyme and the volume of pancreatic juice and of bile. The digesting mixture becomes less acid during its passage through the small intestine.

When the duodenum is filled with chyme, it mixes and propels the intestinal contents by active movements of its walls. The small intestine exhibits peristaltic, segmenting, and pendular muscular movements. The last two are myogenic in nature, since they are not abolished by paralytic drugs. In addition to these gross motions of the small intestine, approximately five million villi are constantly swaying, shortening, and elongating, thereby aiding digestion and absorption. X-ray observations, after barium meals, show that approximately five to seven hours are required for the passage of intestinal contents through the 26 feet of the human small intestine. The progress is most rapid in the duodenum and slowest

in the ileum. As much as two thirds of the human small intestine may be removed surgically without impairment of satisfactory compensatory digestion.

Absorption

Only a small fraction of the duodenal contents reaches the colon; the absorbable portion passes through the mucosa of the small intestine into the blood and lymph. Digestion of colloidal foods produces small chemical units which are water-soluble and diffusible, or which can form diffusible coordination compounds. The digestion of foods is necessary because the normal intestinal epithelium, unlike capillary endothelium, is essentially impermeable to colloids. Only minute traces of proteins, proteoses, or peptones permeate the small intestine, except when the mucosa is abnormal.

Foods are not absorbed readily from the mouth, the esophagus, or the stomach. These organs do not possess the special absorbing mucosa found in the small intestine. Small quantities of certain drugs can be absorbed from the mouth; alcohol, carbon dioxide, oxygen, water, and iron and sodium cations in smaller quantities are absorbed from the stomach. However, the normal digestion products of foods are absorbed only in the small intestine. The normal retention of the digesting mixture in this cavity allows efficient absorption of the diffusible products through the special epithelium. Intestinal absorption decreases aborally, being most rapid in the duodenum. Normally, the isotonic remnants of chyme which reach the ileocecal valve contain only insignificant amounts of digestible carbohydrates, fats, or proteins. In diarrhea, or after taking laxatives, peristalsis is increased, and abnormal conditions prevail.

Intestinal Villi

The columnar absorbing epithelium of the human small intestine is spread over five million slender processes, the intestinal villi, whose combined surface is approximately 12 square yards. Each villus contains a capillary network, a nerve plexus, smooth muscle fibers, and a central lacteal. The villi exhibit rhythmic pumping motions, which are stimulated by the presence of food material or fluid in the intestinal lumen. These movements are controlled reflexly through Meissner's plexus; the latter is stimulated by physostigmine, but not by acetylcholine, or by adrenaline. Auerbach's plexus, which governs gross intestinal movements, is stimulated by acetylcholine. Yeast extracts initiate active movement of the villi; the active substance, *villikin*, is in the Bios fraction (page 665). Hydrochloric acid extracts of duodenal mucosa also contain this hormone; it is not identical with histamine, secretin, or cholecystokin. Other active stimulants of the villi are alanine, bile, histamine, leucine, and phosphate anions. The villi of the human small intestine are capable of pumping 150 ml. of fluid per minute; but the physiological

necessity of this function is not demonstrated, since certain rodents have immobile villi. Another suggested function of the movement of the villi is the evacuation of absorbed fat from the central lacteals. These lymph vessels pass from the villi to valve-containing plexuses which allow only afferent lymph flow from the intestine.

Diffusion Through the Small Intestine

The small intestine allows diffusible food materials and toxic substances to pass with little discrimination. Diffusion of water-soluble substances is the basis of intestinal absorption, and the movement of water through the epithelium follows the laws of osmosis. The intestine does not absorb all diffusible substances at equal rates. It modifies diffusion rates by means of hydrotropic surface-active substances, which combine with certain digestion products to form soluble chemical complexes. It also achieves selective absorption of foods by intracellular chemical reactions, especially phosphorylation and esterification. These chemical transformations remove absorbed substances and increase their diffusion gradients. The chemical processes of the intestinal mucosa are very sensitive to changes in temperature and blood supply, and to mechanical injury. The intestinal *absorption coefficient* for intact animals, as defined by Cori, is the number of grams of food absorbed per 100 grams of body weight per hour. It is affected somewhat by gastric evacuation time.

When a membrane is permeable to a mixture of substances, the direction of passage of any particular component of the mixture depends partially on the chemical nature of the membrane. Incongruent osmosis, that is, diffusion in apparent contradiction to simple conceptions of osmosis, occurs during intestinal absorption because of the influence of constituents in the intestinal contents, the epithelium, and the blood plasma. (Consult Schreinemakers, *Lectures on Osmosis*, for details.) The epithelium of the intestinal wall is a complex colloidal filter whose permeability is affected by the passage of materials; also, the mucosa is a double membrane which shows irreciprocal or unidirectional permeability (page 54).

Hydrotropic Action

Substances which lower surface tension tend to enter cells quickly, and to assist in the absorption of other materials. Intestinal absorption is more specific than this, since certain bile salts form characteristic chemical complexes with the digestion products of fats. Bile salts, therefore, allow more effective absorption of fatty substances than would other substances of equal surface activity. Bile salts, soaps, and certain aromatic acids, which increase the solubility of a variety of substances in water, are termed hydrotropic substances. Solutions of bile salts have a remarkable hydrotropic behavior; they dissolve very insoluble fatty acids, calcium phosphate, calcium carbonate, carotene, cholesterol, vitamins D,

E, and K, and certain hydrocarbons. Bile salts prevent the precipitation of calcium and magnesium soaps in the small intestine, and assist in the absorption of these essential inorganic cations. Extracts of intestinal mucosa, blood and other tissues contain hydrotropic substances other than bile salts.

Bile salts increase the absorption of fatty acids either by affecting the intestinal membrane, or by forming soluble diffusible complexes. Above pH 8, fatty acids tend to exist as soaps, which are the salts of fatty acids with inorganic cations; but between pH 6 and 8, they combine with certain bile salts to form complexes. Crystalline bile acid-fatty acid complexes have been produced *in vitro*. These *choleic acids* are negatively charged and their solutions have a much lower surface tension than do soap solutions. The optimum pH for choleic complex formation is 6.5, the average pH of the duodenal contents; below pH 6, the complexes disintegrate. While choleic acids of the conjugated bile acids have not been isolated, there is evidence that soluble glycocholate complexes, with an average ratio of 1 mol of fatty acid to 3 mols of glycocholate, will diffuse through membranes. Similar reactions of bile salts promote the absorption of antipyretics, camphor, curarine, salicylates, strychnine, typhoid vaccine, and toxins. Unlike most food substances, the bile salts are absorbed least in the duodenum and most rapidly in the ileum, suggesting that they are separated from the fatty acids during passage through the duodenal epithelium. The bile salts tend to remain adsorbed at the epithelial surface of the duodenum, and they assist repeatedly in fatty acid transfer. In this fashion 1 bile salt molecule can readily transport from 8 to 24 molecules of fatty acid after an average meal.

Synthetic Reactions

The living epithelial cells absorb the *d* forms of glucose, galactose, fructose, xylose, and xyloketose more rapidly than other sugars, and at rather constant rates over a wide range of concentration. This rapid selective absorption has been traced to synthetic chemical reactions, especially *phosphorylations*, in the epithelial cells. It has been shown that the first three sugars mentioned are converted to phosphate esters and their diffusion gradients are thereby increased. Other sugars are phosphorylated less easily, and are absorbed more slowly. A similar combination of phosphate with vitamin B₁ (thiamin), vitamin B₂ (riboflavin), and glycerol takes place in the intestinal epithelia during absorption. Since phlorhizin and iodoacetic acid inhibit phosphorylation reactions in living cells, it is not surprising that these poisons interfere with intestinal absorption of glucose, galactose, riboflavin, and fatty acids. Normal thyroid and adrenal cortical function assists absorption in the intestinal mucosa. Decreased absorption in adrenalectomized animals is probably caused by inanition

and disturbances in the $\frac{\text{sodium}}{\text{potassium}}$ ratio; forced feeding and sodium chloride ingestion restore normal glucose absorption in adrenalectomized animals, and in iodoacetate-poisoned rats. Thyroglobulin and normal concentrations of potassium exert a favorable influence on the synthetic chemical reactions and the selective absorption of glucose.

A second synthetic process, *esterification*, occurs in the intestinal epithelium. Glycerol is rapidly esterified with fatty acids to form neutral fats, a process termed the resynthesis of fat. Cholesterol and vitamin A are similarly esterified with fatty acids. Esterification may partly explain why these substances and the sex hormones (sterides) are better absorbed than are the plant sterols. The synthetic reactions considered above are not limited to the intestinal epithelium; they are suspected factors in the subsequent passage of absorbed food substances through capillaries and tissues.

Other Influences

Changes in the permeability of cells are produced by altering the state of imbibition, or swelling of cell colloids, but the exact role of this influence in intestinal absorption is undetermined. Autonomic nervous impulses have small and probably indirect effects on intestinal absorption. They are related to vasomotor changes. The blood flow through the normal intestine is greatly increased during digestion. Circulatory stasis, or ischemia of the intestine, hinders physiological absorption; pathological changes occur in the mucosa, the villi cease their movements and abnormal absorptions ensue.

Absorption of Enzymes, Gases, Etc.

The normal intestine is impermeable to cells and solid particles; however, bacteria and starch grains can penetrate wounded or inflamed mucosa, and are then phagocytized by leukocytes. Gastro-intestinal enzymes and sulfomucin are at least partially digested; some amyllopsin and trypsin pass into the feces. Absorption of intact enzymes from the normal intestine has not been proved. The small traces of amylase, pepsinogen, and prorennin which find their way into the blood and urine seem to enter the blood stream from the secretory glands. When ingested, these enzymes do not appear in the urine unless the intestinal mucosa is abnormal.

The absorption of gases from the small intestine is proportional to their solubility and diffusion. Carbon dioxide and oxygen are rapidly absorbed from both stomach and intestine. Hydrogen is absorbed less readily and nitrogen very slowly. Methane and hydrogen sulfide, formed by bacterial action in the large intestine, are partly absorbed.

THE LARGE INTESTINE

The ileocecal sphincter at the lower end of the small intestine opens at intervals, permitting the passage of fluid into the cecum. It also prevents the free passage of bacteria from the highly infected cecum into the small intestine. The chyme residue requires approximately ten hours to pass through the 5 feet of the large intestine, and it usually remains in the colon another sixteen to twenty-eight hours before defecation. The progress of the fecal mass is affected by the type of food, the functional state of the intestinal muscle, and the rate of water absorption. High fat or carbohydrate diets, foods with a high fiber content, muscular exercise, emotional states, and the administration of pituitrin, acetylcholine, pilocarpine, or physostigmine, stimulate motor activity of the large intestine. Adrenaline and morphine are inhibitory. Atropine relieves intestinal spasm by decreasing muscular tone, but it does not decrease contractions. The augmenting action of the vagus, and the inhibitory effects of sympathetic nerves on intestinal movements, are discussed in physiology texts.

The large intestine normally absorbs about two thirds of the water entering from the small intestine. Nutrient enemas of glucose, amino acids, or sodium chloride are partly absorbed and are of therapeutic value to patients who cannot be fed by mouth. Hydrostatic pressure is a negligible factor in ultrafiltration through the small intestine, except locally during the passage of a peristaltic wave. The large intestine operates as a closed cavity in which hydrostatic pressure is maintained by peristaltic and antiperistaltic contractions. The pressure falls after defecation, and then gradually increases again to provide the driving force for the filtration of isotonic salt or glucose solutions through the epithelium. Concentration of fecal material is, therefore, a very different process from active absorption in the small intestine. In the latter, the individual foods and water are absorbed by osmotic forces modified by special chemical mechanisms; and the solution which leaves the ileum is isotonic. In the colon, the fecal masses are gradually solidified by removal of water and salts; and, when the intrarectal pressure reaches 40 to 50 mm. of mercury, defecation occurs.

Feces

The 400 ml. of semiliquid ileal contents, which daily pass the human ileocecal valve, are concentrated in the cecum and ascending colon to approximately 150 gm. of solid feces. During starvation only 7 to 8 gm. of feces are excreted daily. In quantitative experiments, feces may be separated into colored sections, representing dietary periods, by feeding capsules of charcoal or carmine between periods. Human feces normally contain approximately 75 per cent of water, 4 per cent of fats, 3.5 per cent of inorganic salts, 2 per cent of sterols, and 1 per cent of total nitrogen.

The stools of nursing infants are more watery (85 per cent water). Approximately half of the fecal fat is neutral fat; the rest consists of fatty acids and soaps, principally calcium soaps. The lipide distribution in children's feces is as follows: soaps, 50 per cent; sterols, 30 per cent; and fats and fatty acids, 10 per cent each. The average nitrogen excretion in the feces of the adult is 1.3 gm. daily.

The *exogenous* constituents of feces include such food residues as hemicelluloses, plant sterols, and calcium, iron and magnesium salts. Less than 5 per cent of the digestible dietary nutrients reach the large intestine. The major portion of the fecal material is, therefore, *endogenous* in origin and is excreted by glands of the gastro-intestinal tract. An isolated intestinal loop will accumulate a mass of modified fecal material which contains a large quantity of lipides. The feces of starving patients, and the meconium of the newborn are similar endogenous accumulations. The endogenous fecal output is proportional to the dry weight of the food eaten and is increased by eating indigestible fiber, or hemicellulose.

On an average diet, the daily excretion of endogenous total nitrogen is approximately 0.75 gm. The fat in normal feces is largely endogenous; it is excreted mainly by the intestine, and a smaller portion by the liver. Fecal fat resembles blood or tissue lipides more closely than food fat; it contains appreciable quantities of sterols (dihydrocholesterol and coprosterol) and of phospholipides. The bile and succus entericus excrete calcium, magnesium, iron, copper, and phosphate; the large intestine contributes a smaller quantity of these inorganic substances. The bile pigments appear in feces in their reduced forms, stercobilin and stercobilinogen, which are identical with the urobilin and urobilinogen of urine (page 538). Other endogenous substances found in the feces are sulfomucin, cellular debris, and small quantities of bile salts and of gastro-intestinal enzymes.

The average pH of normal adult feces is near 7, while that of infants is lower (Table 27, page 132) inasmuch as dietary lactose lowers the fecal pH. With ordinary mixed diets, the stool color is brown; with meat diets it becomes darker; with milk, lighter. Calomel, bismuth, and vegetable purgatives impart green, black, and yellow colors, respectively. The disagreeable odor of feces is due to such bacterial products as skatole, indole, mercaptans, and hydrogen sulfide; it is increased by meat diets and decreased by milk. The pH, consistency, color, and odor of feces are related to the bacterial flora of the intestine.

Bacteria in Feces

Micro-organisms are few in number in the small intestine, but they multiply freely in the large intestine, and bacteria usually constitute between 10 and 25 per cent of the fecal mass. The common human fecal bacteria include *Esch. coli*, *A. aerogenes*, *Clostr. welchii*, *Strept. fecalis*, *L.*

bifidus, and *L. acidophilus*. Since bacteria are introduced with the food, the colon of the newborn infant is sterile. Feces of the breast-fed infant contain large numbers of *L. bifidus*, whereas *L. acidophilus* preponderates in the feces of artificially fed infants. The other organisms, and especially *Esch. coli*, are typical of the feces of adults. The bacterial flora increases during gastro-intestinal disorders, or with diets containing indigestible hemicelluloses. Fecal bacteria are, therefore, minimal on a meat diet and maximal on vegetable and fruit diets. The plant foods contain varying amounts of indigestible fiber which serves as food for bacteria. Vegetable diets increase, and may often double, the bulk, total solids, and bacterial content of feces. Increased water intake at mealtime lowers the bacterial count of the feces.

In the large intestine, bacteria produce such gases as hydrogen, carbon dioxide, ammonia, hydrogen sulfide, methane, and mercaptans. The principal foods causing *flatulence* or gas accumulations from bacterial action in the gastro-intestinal tract are: raw apples, beans, broccoli, cabbage, carbonated waters, candies, cauliflower, all highly fermented cheeses, cucumbers, sweet drinks, garlic, jam, meat broths, melons, onions, peas, radishes, syrups, and turnips.

Bacteria in the large intestine create a reducing environment in which the endogenous fecal cholesterol is reduced to coprosterol, and bilirubin is reduced to stercobilin. Bacteria also hydrolyze endogenous fats to fatty acids and soaps; and they change proteins, amino acids, and phospholipides to a variety of amines, phenols, and other so-called *aporrhegas* (Table 67, page 363).

The two chief metabolic activities of intestinal bacteria are classified as (a) *aciduric* or *fermentative*, and (b) *putrefactive*. *L. bifidus* and *L. acidophilus* have pronounced fermentative effects; *A. aerogenes* and butyric acid bacilli also produce organic acids and gases from carbohydrates. The other organisms tend to be putrefactive on high protein diets and to form *aporrhegas* from the partial digestion products of proteins and phospholipides. *Esch. coli*, for example, is classified by bacteriologists as a non-proteolytic organism, but this does not preclude its action on the digestion products of protein. In the presence of sufficient carbohydrate or peptone, *Esch. coli* can also attack proteins.

The metabolic activities of most fecal bacteria are influenced by the available food residues. When carbohydrate is available in the large intestine, as after high carbohydrate diets or administration of lactose, the aciduric bacteria (especially *L. bifidus* and *L. acidophilus*) produce appreciable quantities of lactic, butyric, propionic, acetic, formic, oxalic, and succinic acids by fermentation. As a result, the feces become more acid and fluid; and, in infants, diarrheal conditions may develop. For this reason, pediatricians carefully control the carbohydrate, fat, and protein contents of infant formulae. Lactose and dextrin are rather slowly absorbed in the small intestine, and are most favorable to the growth of

aciduric bacteria. Such sugars encourage the growth of many organisms, but the acids produced during fermentation are unfavorable to the colon group and to putrefactive activity. Growth of *Esch. coli* is inhibited below pH 5. High protein diets favor putrefactive activity, the production of aporrhemas, and the predominance of *Clostr. welchii*, *Strept. fecalis*, *Esch. coli*, and bacteria of the subtilus group.

PATHOLOGY

"Practical men who call loudly for the facts and profess to distrust all theory are only too frequently the last to recognize how much of the so-called facts is just old hardened theory." — MORRIS R. COHEN

SALIVARY PATHOLOGY

A portion of the minerals in saliva is easily precipitated in the presence of bacterial organic products and collects on the tooth surfaces as *tartar*. This insoluble coating consists principally of calcium phosphate, sulfomucin, and cellular and food debris. Bacteria are always present in the oral cavity, but extensive invasion occurs during respiratory and gastro-intestinal infections. The suppression of salivary secretion which accompanies fevers and other anhydremic conditions encourages greater bacterial activity in the mouth and throat and results in foul breath. Occasionally, pathological deposits of calcium salts are found in the salivary ducts. These calculi are subject to the same influences as are other pathological calcifications (page 630).

Mercury, lead, fluoride, iodide, and the viruses of rabies and poliomyelitis are partially excreted in saliva. The salivary excretion of mercuric ions, in mercury poisoning, produces severe inflammation of the mouth (stomatitis). The blue lines which appear at the margins of the gums in lead poisoning are due to deposits of lead sulfide, produced by the action of sulfides generated by the tartar bacteria. The presence of small excesses of fluoride in the water supply causes pathology of the enamel which results in *tooth mottling* (page 635). In nephritis, urea increases in the blood stream, and this diffusible substance also appears in the saliva in abnormally high concentrations. The stomatitis of nephritic patients is caused by ammonia, which is liberated from urea by the action of bacterial ureases. *Glossitis*, or sore tongue, appears in pernicious anemia, sprue, and pellagra.

Complete lack of saliva, *aptyalism*, is seldom encountered in the human being; but excessive salivation is frequently produced, reflexly, by duodenal ulcer, esophageal and oral lesions, gastritis, pregnancy, and by the introduction of gastric or duodenal tubes.

In *dental caries*, the tooth enamel breaks and the underlying dentine is softened. This condition is most prevalent between the ages of ten and thirty years. The relation of saliva to dental caries is not definitely estab-

lished. While careful brushing of the teeth is a desirable hygienic measure, it does not always prevent caries. Bacteria, by producing organic acids and other calcium-dissolving substances, undoubtedly assist caries development; but lowered salivary pH is not as fundamental as is sometimes claimed. Pregnancy, hereditary influences, and deficiencies of calcium, phosphate, fluoride, and vitamins A, C, and D during childhood are important predisposing factors. The unfavorable influence of high carbohydrate diets may be due partly to replacement of essential foods. Candies, desserts, and other concentrated sugar preparations have a high satiety value and should, therefore, be taken after meals. It is customary to place the dessert course at the end of the meal where it does not have an unfavorable influence on food selection. Odontoblasts are especially sensitive to lack of ascorbic acid and to vitamin A deficiency. Vitamin D is also concerned in the proper calcification of teeth. Adequate fluoride intake has an inhibitory influence on the development of dental caries, but its uncontrolled prophylactic use is complicated by the tendency to produce mottled enamel (page 635).

GASTRIC ANALYSIS

The physician is particularly interested in the composition of gastric contents following the administration of a standard test meal, although occasionally gastric juice is analyzed to determine the extent of neutralization during alkali therapy of peptic ulcer. The most popular test meal is the *Ewald meal*, which consists of two slices of unbuttered toast (or other cereal substitute) and 8 ounces of water or weak tea. The analytical samples are obtained by means of a gastric tube. The residual gastric juice is withdrawn and its volume noted. After a night's fast, there are normally 50 ml. or less of residual juice, the result of a continuous slight secretion. If the volume is much larger, or if the residual juice contains remnants of food, abnormal retention is indicated. After the residuum has been removed, the test meal is given; and one hour later, according to the original Ewald method, the gastric contents are completely aspirated. Some clinicians prefer a fractional procedure in which 5 ml. of gastric contents are withdrawn, at fifteen or thirty minute intervals, during a two hour period. The fractional method provides curves of gastric response rather than empirical one hour values. The stomach contents should be mixed *in situ* before withdrawing fractional gastric samples.

Free hydrochloric acid is determined in filtered gastric samples by titration with 0.1 N sodium hydroxide. With Töpfer's reagent (dimethylaminoazobenzene) as indicator, a salmon pink endpoint is obtained at pH 2.8 (Table 5, page 17). Phenolphthalein is then added to the mixture and the titration is continued to the endpoint of this indicator, in order to determine the total acid. The results are usually expressed as "units" or

"degrees" of acid, that is, ml. of 0.1 N acid per 100 ml. of gastric contents. The pH is determined in a separate small aliquot of filtered stomach contents, with the colorimetric procedure and an alcoholic solution of thymol blue as indicator (pH range, 1.2 to 3.0). When free hydrochloric acid is absent, the pepsin content is sometimes determined. There are several ways in which this can be done; for example (1) by measuring the amount of dye liberated from stained fibrin, (2) by colorimetric determination of hemoglobin digestion, (3) by the digestion of coagulated albumin in Mett tubes, (4) by determination of the degree of digestion of suitable substrates by Sørensen's formol titration, Van Slyke's amino nitrogen method, or estimation of incoagulable nitrogen.

Microscopic examinations and tests for blood are customary. In peptic ulcer, the gastric contents frequently contain bright red blood; while in gastric carcinoma, brown-black "coffee-grounds" blood (blood which has been coagulated and converted to acid hematin) is present. The emptying time of the stomach may be estimated by the disappearance of food particles from the aspirated samples, or by iodine tests for starch. The detection of lactic acid is especially important; the ferric chloride test is generally preferred for this purpose. Since lactic acid is a bacterial metabolite originating from dietary carbohydrates, it is present in gastric contents in conditions of motor deficiency, retention, and hypochlorhydria. In the absence of abnormal quantities of lactic or butyric acid, the total acid represents the secretory efficiency of the gastric glands in response to the test meal.

The response of the normal stomach to the Ewald meal at one hour is as follows: volume, 50 to 100 ml.; total acid, 60 ± 15 units; free hydrochloric acid, 40 ± 10 units; pH, 1.7 ± 0.1 . The pepsin content usually parallels the total acid. By means of the fractional method it has been shown that both free and total acidity normally increase to a maximum at one hour, remain at this level for about thirty minutes, and then decrease to the original values in from two to two and one-half hours (at which time the stomach has been evacuated). If the test diet is changed to one containing meat, the maximal values for both free hydrochloric and total acid are approximately doubled (Table 28, page 140). The secretory response to the Ewald meal is somewhat less in children and in females than it is in adult males; after middle age it decreases progressively with age.

The subcutaneous injection of 0.01 mg. histamine hydrochloride per kg. of body weight, or a meal of 50 ml. of 7 per cent alcohol, provides a useful estimate of gastric secretion. Maximal response of the normal adult to the standard histamine injection is usually obtained in from thirty to forty-five minutes. The maximal ten minute volume is approximately 37 ml. of juice at the age of twenty-five, decreasing to 24 ml. at the age of sixty-five. The corresponding maximal total acidities are approximately 90 and 67 units, respectively.

Interpretation of Gastric Analysis

In conjunction with x-ray studies and general diagnostic procedures, the gastric acidity data are important for the detection and differentiation of pathological gastric processes. However, the wide physiological variations encountered in normal persons require that the laboratory findings be interpreted in accordance with clinical findings. Repetition of the tests on different days adds greater weight to the laboratory evidence.

Certain gastric symptoms are traceable to disturbances of gastro-intestinal motility. Thus, pyloric stenosis and rapid emptying of the stomach induce characteristic symptoms. Motility disturbances are closely related to the character of the food and to chemical digestive processes. The intimate relations of psychic factors to gastric symptoms have been amply demonstrated. Nervousness and anxiety increase peristalsis, while mental strain, fear, depression, pain, and fatigue inhibit it.

The gastric tests show two general pathological tendencies, namely, hypofunction and hyperfunction of the gastric glands. The latter represents increased volume of gastric secretion.

HYPOFUNCTION

Abnormally low acidity of the gastric contents after a test meal is called *hypoacidity* or *hypochlorhydria*, depending on whether the total acid or the free hydrochloric acid is low. In hypochlorhydria, the free hydrochloric acid is persistently below 20 units and the pH is above 2.0. The terms *anacidity* and *achlorhydria* denote complete suppression of acid secretion, and the pH is 3.0 or higher. In these cases, qualitative tests for lactic acid should always be made. The entire gastric secretion is usually suppressed in the atrophic condition of the gastric glands — *achylia gastrica*. The histamine test and the determination of total chloride in gastric juice are particularly useful in differentiating achylia from anacidity or achlorhydria. Test meals are used to differentiate these conditions, since there are instances in which the glands respond more to a meat diet than to histamine injection. Frequently, however, histamine has a secretagogue effect where test meals fail, and the response to this stimulant is not markedly affected by psychic influences. At times achylia is fully compensated by intestinal digestion. Most of the stomach can be removed surgically without causing extreme digestive inconvenience, provided that proper diets are given.

Hypochlorhydria is frequently associated with rapid emptying of the stomach. About one fifth of otherwise normal individuals have hypochlorhydria, due either to deficient secretion, or to excessive neutralization of hydrochloric acid following its secretion. The total acidity of gastric contents serves to differentiate these types. A small percentage of healthy adults exhibit marked gastric hypofunction; from 3 to 6 per

cent have achlorhydria, and 1 per cent show anacidity. With increasing age, there is a higher incidence of gastric hypofunction. At the age of sixty-five, a 25 to 30 per cent incidence of anacidity is found. Hypoacidity and anacidity develop in pernicious anemia, the anemias of pregnancy, leukemia, gastric carcinoma, advanced chronic gastritis, neuroses of the hyposthenic type, gallbladder disease, malnutrition, nephritis, diabetes mellitus, pellagra, sprue, Addison's disease, scurvy, myxedema, hyperthyroidism, tuberculosis, generalized arteriosclerosis, chronic arthritis, and temporarily during fevers. Gastric adenocarcinoma tissue exhibits little secretory activity. Approximately three fourths of pregnant women have hypochlorhydria and an associated tendency to anemia. Hypofunction at times follows gastro-enterostomy. It is especially frequent after surgical resections of portions of the stomach. In these cases the gastric function usually returns to normal in the course of several years. Achlorhydria and anacidity are constant symptoms of pernicious anemia, and achylia gastrica is frequent. Achylia also occurs in subacute combined degeneration of the spinal cord, and after gastro-enteritis in children. It is not relieved by histamine injections, and may require hydrochloric acid therapy. The role played by hydrochloric acid in the absorption of iron is considered elsewhere (page 603). Persistent diarrhea, which is a symptom of anacidity, responds to the administration of dilute hydrochloric acid.

The *dietary treatment* of hypofunction includes the ingestion of dilute hydrochloric acid, or of glutamic acid hydrochloride. Small quantities of alcoholic stimulants are sometimes given, but greater reliance is generally placed on such natural gastric stimulants as meat and fruit juices. Easily digestible foods are provided; excessive proportions of fats, which are inhibitory to gastric secretion, or of fermentable sugars, which encourage bacterial growth, are avoided.

HYPERFUNCTION

Approximately 5 per cent of healthy adults exhibit *hyperchlorhydria* or hyperacidity by the Ewald test meal; in some cases the fractional curves, after the one and one-half hour period, continue to rise and maintain temporary high levels. It is important to realize that hyperchlorhydria represents an increased secretory response of the glands to the test meal, and not the secretion of gastric juice with an abnormally high acid content. At times, hyperacidity is the result of delayed evacuation traceable to pylorospasm or to pyloric obstruction. Gastric retention tends to provoke vomiting, alkalosis, and tetany. Because of pylorospasm and reflex stimulation of the gastric mucosa, hyperacidity is a frequent symptom of ulcers near the pylorus and of gastric stenosis. It is also encountered in neuroses of the hypersthenic type, early gastritis, gallbladder disease, and appendicitis, where it is often of reflex nervous origin. An important clinical

symptom of hyperacidity is pain which occurs in the case of gastric ulcer within one and one-half hours after meals, and at a later period with duodenal ulcer. The pain is quickly relieved by the administration of food or alkali. Determinations of the acidity of the gastric contents after a test meal of 300 ml. of 0.5 per cent hydrochloric acid solution have been used to study duodenal ulcer. Decreasing acidity in successive samples indicates improvement in the gastric situation. In normal persons, the total acid decreases to approximately 40 units in one to one and one-half hours, and the pH to 1.4. The *dietary treatment* of hyperacidity is similar to that of gastritis.

GASTRITIS

This inflammatory condition is produced by infections, dietary errors, alcohol, or drugs. It is often secondary to psychoneuroses, or to gall-bladder, liver, heart, or kidney disease. The gastric contents exhibit an excessive sulfomucin content. In *acute gastritis*, chemical examination is limited to the vomitus, and food is often withheld for a day or two. The subsequent treatment includes rest and small feedings of *non-irritating foods*, such as milk, eggs, fruit juices, cereals, and toast, which constitute a "bland diet." In *chronic gastritis* with hypofunction, a normal diet and either thiamin or vitamin B complex are administered; while in gastritis with hyperacidity, cream and fat are incorporated into the diet to inhibit gastric secretion.

GASTRIC AND DUODENAL ULCERS

Peptic ulcers have important relations to gastric acidity. The chief factor in the progress of peptic ulcers is the digestive action of gastric juice on the damaged tissue, and this is related to both the hydrochloric acid and the pepsin content. Ulcers appear most frequently in regions of the gastro-intestinal tract where hydrochloric acid remains unneutralized for some time, as in the pyloric portion of the stomach, in the upper portion of the duodenum, and, after operation, in the region of the anastomosis of the intestine and stomach. They also form occasionally in the cardia and in the lower end of the esophagus. The acid-secreting regions of the normal stomach apparently have protective mechanisms which prevent this condition.

The hyperirritability of the ulcerated stomach causes hyperacidity both during and between meals. When gastric motility is also increased, the fractional test shows a decline in acid after the first hour and one half; otherwise the acidity remains high, giving what is known as a plateau type of curve. With spasm of the pylorus, the acidity is still mounting at two and one-half hours. Gastric ulcers lead to less hyperacidity than do duodenal ulcers, probably because the former tend to produce an associ-

ated gastritis. Caffeine and the beverages which contain it (coffee, cola beverages) stimulate excessive gastric secretion in peptic ulcer patients.

The prevention of excessive gastric acidity is important in the treatment of peptic ulcers, and in the prevention of recurrence following operation. That factors other than hydrochloric acid are concerned in initiating the pathological process is obvious from the fact that in many cases of hyperchlorhydria ulcers are absent. Normal gastric mucosa and abdominal organs sutured into gaps made in the stomach wall are not digested by gastric juice. Similarly exposed limbs and ears are digested, and the reasons for these differences are obscure. Circulatory, bacterial, and neurogenic theories have been proposed to explain ulcer production. Neurogenic factors have definite relations to the progress, if not to the inception, of ulcers. Experimental chronic ulcers can be produced in dogs by the administration of cinchophen, or by intramuscular injection of histamine in a beeswax-mineral oil mixture (which allows a prolonged stimulatory action of the histamine). The occurrence of Curling's ulcer after severe burns is preceded by marked stimulation of gastric secretion.

Treatment

Modern treatment of peptic ulcers is directed toward improvement of the disturbed gastric equilibria. To maintain adequate nutrition with least irritation, the physician orders frequent small meals of good buffer value and high fat content, which do not stimulate oversecretion of gastric juice. The foods used include milk, cream, butter, and eggs (various modifications of the Sippy diet). It is important to limit the intake of meat and other stimulants to gastric secretion as well as of mechanically irritating fibrous vegetables. In addition, controlled alkali therapy is employed. To avoid alkalosis, the less readily absorbable alkalis, such as aluminum hydroxide, aluminum phosphate, aluminum silicate, calcium carbonate, tricalcium phosphate, magnesium oxide, magnesium phosphate, and magnesium trisilicate are frequently preferred to sodium bicarbonate. Atropine medication and supervision of the psychic state are additional means of preventing oversecretion of gastric juice. In the future, enterogastrone may be used in the treatment of peptic ulcer. Patients on the Sippy diet readily develop ascorbic acid (vitamin C) and thiamin (vitamin B₁) deficiencies. These vitamins should be administered as prophylaxis against severe hemorrhage and development of neuritic symptoms.

PANCREATIC PATHOLOGY

In *chronic pancreatitis*, the pancreatic juice has deficient digestive powers; the feces contain portions of undigested fat, protein, and carbohydrate. The stools are soft, bulky (1000 gm. daily), and pale in color from excess fat. Microscopic examination reveals undigested fat, muscle fibers (creatorrhea), starch, fatty acids, and soaps. Schmidt's test diet may be

given to provide standard conditions for the fecal examination. Pancreatic insufficiency differs from other steatorrheic conditions in that it is accompanied by markedly decreased absorption of nitrogenous substances, with loss of as much as 80 per cent of the protein intake. Hence, azotorrhea exceeding 3 to 4 gm. of fecal nitrogen per day, on the Schmidt test diet, is an important diagnostic sign.

In *acute pancreatitis*, which follows obstruction of the pancreatic duct, there is abdominal pain, vomiting, and shock which may rapidly prove fatal. In the severe hemorrhagic form of the disease, the gland undergoes necrosis and autodigestion by trypsin. Steapsin may escape from the injured gland into the abdominal cavity and cause *fat necrosis* of the mesentery, omentum, peritoneum, and adjacent organs. Interstitial tissue of the pancreas, the tissues of the abdominal area, and leukocytes can activate trypsinogen, causing abdominal *protein necrosis*.

In studies of pancreatitis, the pancreatic enzymes are sometimes determined in samples of duodenal contents secured through a duodenal tube. Trypsin is estimated by the digestion of fibrin stained with Congo red, or by turbidity determinations of casein digestion. Lipase is determined by titration of the fatty acids liberated from olive oil, and amylase by the amounts of reducing sugar formed from starch. Normal urine contains from 3 to 30 units of amylase (Wohlgemuth's method), which increases to 100 or more during acute pancreatitis. Normal plasma amylase is between 80 and 150 units (Somogyi method). It is raised markedly in acute pancreatitis and after occlusion of the salivary duct, and is lowered in diabetic ketosis and liver disease. The diagnostic value of increased blood and urine amylase is rather empirical; these amylases are not derived solely from the pancreas. Blood amylase is also elevated, at times, in diabetes, gastritis, high intestinal obstruction, mumps, peptic ulcers, pneumonia, rachitis, renal damage, starvation, and typhoid fever. Pancreatitis is one of the complications of mumps (parotitis). Lipase increases in the blood in acute pancreatitis, pancreatic carcinoma, and, occasionally, after hepatic injuries; normal blood contains esterase but little lipase.

Complete *drainage of the pancreatic juice*, through external fistulae, is fatal in a week or two. Dehydration and acidosis from loss of body fluid and base are partly responsible for death, but intravenous saline does not permanently remedy the situation. The fatal outcome is not due to loss of digestive functions; for, in the absence of pancreatic juice, a carefully selected diet is partially digested by enzymes of the succus entericus and the intestinal mucosa. One authority estimates that three fourths of the milk fat, two thirds of the dietary protein, and most of the dietary carbohydrate are digested. Even though certain fats are partially digested, they are poorly absorbed, and the difficultly digestible fats are lost entirely in the feces.

In the *dietary treatment* of chronic pancreatitis, rigid limitation of fat intake is practiced. Meats are withheld when excess muscle fibers are

found in the feces. Milk, eggs, and toast form the chief components of the diet, which should be high in protein; pancreatin (a preparation containing pancreatic enzymes) may be given to assist digestion, or protein hydrolyzates can be administered.

BILIARY PATHOLOGY

When normal quantities of bile are prevented from reaching the small intestine, as in *obstructive jaundice*, dietary fats are poorly absorbed and endogenous lipide excretion is increased. Fats and fatty acids then appear in large quantities in the feces despite digestion by steapsin and intestinal lipase. Significant amounts of calcium, derived from the intestinal secretions, are lost in the feces (chiefly, as calcium soaps of fatty acids). Obstructive jaundice is caused by gallstones, inflammation, infection, or by tumors of the gallbladder or biliary passages. When these processes involve the gallbladder, its concentrating ability is diminished and bile salts are reabsorbed. In obstruction of the common bile duct, the bladder retains its absorbing ability and may, in time, become filled with a thin colorless secretion derived from the bile ducts; this condition is known as *hydrops* of the gallbladder. Another type of "white bile" is produced by severely damaged or atrophied liver parenchyma which can no longer excrete bile pigments. Bile secretion is reduced by such pathological conditions as fevers, cachexia, and hepatic disease. The pressure-regulating mechanism of the gallbladder delays the appearance of jaundice (bile pigment accumulation in the general tissues) for two or three days following biliary obstruction.

A number of the symptoms of gallbladder disease are reflex phenomena of the stomach and intestine. Anacidity, hyperacidity, and colitis are at times traceable to disease of the biliary tract. Cholecystectomy is frequently performed in cases of biliary calculi and cholecystitis. Following this operation, the delivery of bile to the small intestine becomes nearly continuous; dietary adjustments may be required for a year or two before the ducts dilate sufficiently to permit storage and intermittent evacuation of bile.

Animals with total external *biliary fistulae* develop a characteristic anemia, cachexia, a bleeding tendency, due to inadequate absorption of vitamin K, and osseous abnormalities associated with decreased vitamin D absorption and with loss of calcium in the bile. Permanent fistulae of this type are fatal within from two to ten months, depending on the diet. High carbohydrate diets, milk, and bile administration prolong the lives of such animals.

When bile escapes into the peritoneal cavity, it produces severe inflammation, secondary surgical shock, hemorrhage, necrosis, and *cholemia* (an accumulation of biliary constituents in the blood and tissues). This condition is known as *bile peritonitis* or *cholascos*.

Movements of the gallbladder can be detected by fluoroscopic examination when sodium tetraiodophenolphthalein is administered, for it accumulates in the gallbladder and is opaque to x-rays. This dye, or a similar bromine derivative, may be given either *per os* or intravenously. In a fasting patient, the roentgenogram of the normal gallbladder shows a well defined shadow which disappears in a second picture taken five hours after a meal of cream and egg yolk. Diseased gallbladders do not concentrate the dye effectively and the shadow becomes faint. Emptying of the gallbladder may be inhibited by blockage of the ducts, a condition which can be confirmed by use of the duodenal tube. The functional gallbladder is evacuated by giving olive oil or magnesium sulfate through a duodenal tube. Biliary calculi, which have been discussed on page 76, sometimes contain sufficient calcium to produce shadows in the roentgenogram; usually, however, they are demonstrable merely as relatively light spots in the dye shadow.

Dietary Treatment

Patients with gallbladder disease are provided with a bland, low residue, high carbohydrate diet. In acute cholecystitis, fats (other than cream, butter, and egg yolk), meats, and acid fruits are limited because they stimulate gastrin and cholecystokinin production. Vegetables and fruits which cause flatulence are also avoided (see list on page 154). A meat diet is objectionable in liver or gallbladder disease, because it favors putrefaction and tends to increase ascites. The protein requirements of these patients are best satisfied by dairy products. Bile salts are given by mouth whenever it is desired to promote bile flow or absorption of fatty acids, carotene, and vitamins D, E, and K.

Liver Function Tests

The clinical significance of liver function tests is limited by the great regenerative powers of the liver, its high functional reserve, and the association of hepatic functions with those of other organs. Under optimal conditions, one seventh of the liver is sufficient to perform many of the normal hepatic metabolic functions. Liver function is, therefore, more abnormal during acute and diffuse involvements of hepatic tissue than in slow, chronic liver pathology. The functional tests are particularly useful in preoperative studies of patients.

The determination of serum bilirubin by the van den Bergh method and the icteric index serve to detect accumulations of bile pigment and deficient excretory ability of the liver (pages 537 and 538). The galactose tolerance test (page 338) is used occasionally to aid in distinguishing obstructive jaundice from parenchymal liver disease. The hippuric acid test (page 431) is preferred for the investigation of parenchymatous hepatic function. Inability to remedy hypoprothrombinemia by the injection of

2-methyl-1,4-naphthoquinone reflects extensive hepatic damage (page 67). The urobilinogen content of the urine assists in deciding whether or not the obstruction is complete. Urinary urobilinogen output is increased during blood destruction, liver disease, or infection or partial obstruction of the biliary tract. When the obstruction is complete, urobilinogen disappears from the urine. The metabolism and pathology of the bile pigments are discussed in detail on pages 547 and 558.

Other hepatic tests are based on the rate of excretion of intravenously injected bromsulfalein (2 mg. per kg., in 5 per cent solution), or of bilirubin (1 mg. per kg.), as estimated from the plasma levels of these pigments. The normal liver excretes the bromsulfalein test dose in thirty minutes, and the bilirubin test dose in four hours. Both procedures show decreased excretion during obstructive jaundice and parenchymatous liver damage. They are most useful, clinically, in the absence of demonstrable obstructive jaundice, which in itself indicates excretory incapacity. The rate of disappearance of bromsulfalein from the blood is partly determined by the activity of the reticulo-endothelial system. (See page 558 for further details.)

FOOD ALLERGY

Food allergies give rise to a variety of gastro-intestinal, asthmatic, cutaneous, and nervous symptoms which usually are temporary. Severe food allergy can produce shock, suffocation, or collapse. These phenomena are elicited by the absorption of traces of undigested proteins. It has been demonstrated repeatedly that minute quantities of protein can be absorbed both in the small intestine and in the mucosa of the respiratory and upper digestive tracts. Traces of ovalbumin (from egg white) are absorbed rather easily by many persons. The presence of antitrypsin in egg white may be partly responsible for this result. Infants and patients with hypoacidity are especially susceptible to intestinal absorption of ovalbumin. Allergic individuals are sensitive to one or to a small group of specific proteins, but it is not strictly necessary that undigested proteins be absorbed into the blood stream in order to produce allergic symptoms. In contact allergies, the application of a specific protein to the respiratory or gastro-intestinal mucosa or to the skin elicits an allergic response. There is evidence that the traces of foreign proteins which pass through the small intestine are transported by the lymphatic system. The intestinal epithelium contains large numbers of leukocytes which are attracted chemotactically by food proteins, or by their digestion products. Leukocytes have protective, immune, and phagocytic functions which are discussed on page 487.

Eggs, wheat, and milk are the most general incitants of food allergies; but the proteins of vegetables, fruits, and fish are responsible for other cases. It is the physician's problem to discover the offending foods by

means of skin tests, elimination diets, and other appropriate tests. Skin testing has definite diagnostic limitations, since the skin and the intestinal mucosa are not always equally sensitive to the same proteins. Successful treatment of food allergies requires avoidance of the responsible foods, or desensitization of the patient by frequent oral or subcutaneous subtoxic doses of the offending protein or polysaccharide allergen. Adrenaline and ephedrine temporarily alleviate the symptoms of allergy. The general metabolic aspects of allergic conditions are discussed on page 490.

INTESTINAL OBSTRUCTION

This condition results from adhesions, kinking, intussusception, hernial strangulation, tumors, or disturbed motility of the intestine. It causes characteristic symptoms, including severe abdominal pain, vomiting, occasional alkalosis, oliguria, profound depression, and shock. The vomiting leads to dehydration, alkalosis, and lowering of the blood chlorides (page 42). Reverse peristalsis occurs and it renders the vomitus fecal in character. Tissue destruction and terminal renal insufficiency are reflected by increased non-protein nitrogen concentration of the blood. The portion of the intestine above the obstruction dilates and becomes filled with fluid and gas. *Paralytic ileus* is a functional type of intestinal obstruction which accompanies peritonitis and follows surgical trauma of the intestine. The paralysis of intestinal musculature which occurs is maintained by sympathetic impulses. This condition resembles the atony and dilatation following mechanical obstruction. It can be avoided by early operation. Obstructions of the upper portion of the small intestine cause the most severe symptoms.

Death is not due to the mechanical effects of the obstruction *per se*, for even obstructed isolated intestinal loops can be fatal. Certain investigators believe that toxic proteoses are formed in the obstructed intestine; and it is generally agreed that dehydration plays an important role. The loss of body chloride through vomiting and accumulation of fluid in the dilating intestine hastens death. Plasma chloride may fall to one half its normal value of 365 mg. per cent. During the first day or two, the blood bicarbonate rises as a result of the chloride loss. The alkalosis is accompanied by further losses of cations and anions in the urine, and the anhydremic condition is aggravated. Injection of saline solution definitely prolongs life, but surgical intervention is frequently necessary.

In dogs, total drainage of gastric juice produces symptoms similar to those of intestinal obstruction. The life of an animal with experimental high intestinal obstruction may be prolonged considerably by the injection of the vomitus through a jejunal fistula. However, experimental dilatation of the intestine can produce death without serious chloride loss, and little understood nervous mechanisms may be concerned in the fatal effects of intestinal obstruction.

In rare instances, diseased or obstructed intestines are found to contain *enteroliths* or choleic acid calculi. Foreign bodies in the stomach are termed *bezoars*. They consist of insoluble and indigestible substances, such as hair or vegetable fibers, which, at times, become calcified. The ingestion of unripe persimmons is a frequent cause of gastric bezoars composed of shibuol, a pectin-like substance which coagulates at the pH of the gastric contents.

DIARRHEA

Diarrhea may be caused by an improper diet, gastro-intestinal infections, ulcers, tumors, allergy, neurogenic disturbances, psychoneuroses, hyperthyroidism, pancreatitis, uremia, achlorhydria, and by irritating drugs, such as arsenic, mercury, and iodides. The great majority of acute diarrheas are traceable to invasions of unidentified bacteria; while certain dysenteries are due to amebiasis, cholera, tuberculosis, and typhoid. Sulfadiazine, sulfaguanidine, and sulfasuxidine have been used effectively in the treatment of acute and chronic bacillary dysenteries. Diarrhea is accompanied by increased peristalsis, hypersecretion of succus entericus, decreased absorption, acidosis, and dehydration.

Dietary Treatment

Because of the intestinal inflammation and irritation, diarrhea patients are given non-irritating diets which tend to inhibit intestinal motility. At times, castor oil is administered at the beginning of the treatment of infectious diarrheas, and food is withheld for a day or two while the patient is resting in bed. The first food given is liquid, or semiliquid, and this is followed by a bland diet. In chronic diarrhea, the prevention of dehydration requires the administration of considerable fluid, either by mouth or parenterally (page 39). Milk, broths, orange juice, and tea assist in providing fluid. Because of the bacterial relations (page 154), the diet should be high in protein and low in carbohydrate, unless the diarrhea is complicated by marked putrefaction. Putrefactive diarrhea is normally associated with alkaline stools. Nervous diarrhea is often accompanied by circulatory disturbances in the intestine; in these cases diet is less important than rest, treatment of the nervous instability with thiamin, and similar measures.

Infants are particularly susceptible to diarrhea. The infant's digestive tract operates near functional capacity, hence poorly digested food can easily accumulate in the intestine and serve as a medium for bacterial growth. Absorption of fats is especially poor in premature infants. The gastric juice of infants is only slightly acid (Table 27, page 132) and is not as inhibitory to bacterial growth as that of adults. Neither is intestinal immunity to bacterial invasion as well developed as in the adult. Infants may be protected against diarrhea, especially during warm weather, by

providing properly sterilized and adequately balanced formulae. The presence of excess fat or of fermentable sugar may incite diarrhea. Unfermentable sugars, such as dextrins (karo syrup) and pectins (apple powder) are therefore used. Since lactic acid milk is easily digested and provides a poor medium for certain intestinal bacteria, it is a desirable infant food during acute diarrhea. Ordinary milk is well tolerated by adult patients, but, at times, they are also given lactose and *L. acidophilus* milk. Nicotinic acid (a vitamin of the B complex) has been reported as being beneficial in infant diarrhea.

TYPHOID FEVER

In this disease there is an involvement of Peyer's patches with subsequent ulceration of the intestine. A high caloric, high carbohydrate, non-irritating diet (administered as small fluid feedings, every two hours) is the basis of the *dietary treatment*. Since the caloric requirement of the patient may be doubled by fever or wasting, 40 calories per kg. of body weight should be provided in the diet. Excess carbohydrate tends to reduce the liberation of toxic products by the infecting organism. Milk is unquestionably the most appropriate natural food for typhoid patients. Lactose, cream, eggs, butter, toast, and fruit juices are important constituents of the typhoid diet.

PELLAGRA, SPRUE, AND CELIAC DISEASE

Sprue and pellagra are deficiency diseases of tropical and semitropical climates. The gastro-intestinal symptoms common to these diseases are: stomatitis, glossitis, and, in advanced stages, diarrhea. Since achlorhydria and achylia are often present, the administration of hydrochloric acid is occasionally desirable. Acute cases of *pellagra* readily respond to vitamin therapy (nicotinic acid, other vitamins of the B complex, yeast, and liver extracts), and to high protein diets which contain milk, meat, and eggs. Nicotinic acid is especially efficacious in healing the lesions of the mucous membranes. (See page 661 for general discussion of pellagra.)

The classical form of *sprue* is a tropical dysentery, but it is known to occur in the southern United States. It has several characteristics in common with pernicious anemia, and liver extracts are used in its treatment. Only about 20 per cent of sprue patients have an acidity, but diarrhea is a prominent symptom of the disease. Both fat and calcium are poorly absorbed in the intestine and are excreted in increased amounts in the bulky feces. The alimentary tract shows inflammatory and degenerative lesions. A high protein, low fat diet is given, together with fruits (especially fresh strawberries and ripe bananas), vegetables, milk, and liver. Non-tropical sprue, idiopathic steatorrhea or *celiac disease* resembles the tropical form and may be classified tentatively with this deficiency

disease. In celiac disease, the calcium loss leads to skeletal changes and low serum calcium or phosphorus, with occasional tetany, rachitis, or osteomalacia. Dietary fat is restricted, and vitamin B complex and liver extract are administered.

The high protein diets used in the treatment of these deficiency diseases assist in correcting the diarrheal condition and in the maintenance of body weight; but the main therapeutic objective is to supply sufficient vitamins of the B complex to alleviate the acute symptoms (page 662).

COLITIS

Mucous colitis, of unknown etiology, occurs in certain very nervous patients. Enormous amounts of mucus may be secreted by the large intestine. In this disease, diarrhea alternates with spastic constipation. Bland diets are used in the treatment. For *ulcerative colitis*, which is often due to infections that may be treated by sulfadiazine, sulfaguanidine, or sulfasuxidine, the preferred diet is bland, high caloric, high protein, and high vitamin (brewer's yeast, orange juice, and cod or halibut liver oils). Fruits and vegetables are avoided because of their peristaltic effects. Liver extracts and vitamin B complex are useful in this condition. *Mercury poisoning* produces colitis and lesions of the large intestine. The mercuric salts, excreted in the gastro-intestinal tract, exert a marked corrosive action when they reach the colon.

CONSTIPATION

Constipation is occasionally caused by intestinal obstruction; but in the great majority of cases it is the result of poor dietary and hygienic habits, or of functional abnormalities of the intestinal musculature. Many cases of constipation are of dietary origin, related to deficiencies of fiber, minerals, or fluid. Constipation is, at times, traceable to abnormal absorption of water by the large intestine, or to hypertonicity of the colonic musculature (*spastic colon*). The latter is often of reflex origin from a diseased gallbladder, appendix, or other related organ. It may also result from worry, shock, or overwork. The spastic musculature is relaxed by atropine. There is an *atonic type* of constipation with depressed muscular function associated with obesity, senility, or thiamin deficit. *Megacolon* is a rare condition of disturbed nervous control in children which results in great dilatation and hypertrophy of the colon.

Dietary Treatment

To counteract the constipation resulting from small fecal bulk and lack of moisture, indigestible agar and mineral oil are useful in emergencies. Dietary regulation has proved a more satisfactory treatment of constipation, especially in chronic cases. To assure the necessary fecal

bulk, fibrous fruits, whole grain cereals, and vegetables are incorporated into the diet, together with sufficient water and carbohydrate. Caution is often necessary in the use of bran because it may prove irritating, especially in spastic cases. Cream, butter, and vegetable oils are of additional assistance in changing the character of the feces; the fatty acids liberated from these foods stimulate the mucous membrane. However, if the fat content of the diet is too high, the presence of undigested fat in the large intestine tends to irritate the mucosa.

A normal semisolid stool best serves the functional activity of the large intestine. Concentrated dry fecal masses are irritating to the colonic mucous membrane and tend to remain in the haustra. Hemicelluloses modify bacterial metabolism and are necessary for the formation of the ideal type of feces for human beings. A diet which contains suitable quantities of whole grain cereal products, fibrous and leafy vegetables, and fresh fruits provides the necessary hemicelluloses (Table 59, page 300). Cow's milk, without added carbohydrate, tends to be constipating, especially in artificially fed infants.

BACTERIAL RELATIONS

The normal flora of the small intestine has a predominant fermentative activity, while the flora of the large intestine has a greater putrefactive action. The large intestine also possesses greater immunity to bacterial toxins and aporrhegas than the small intestine. The fermenting organisms ordinarily create a rather unfavorable environment for putrefactive organisms of the colon group. In many cases, the return of a normal fermenting flora to the small intestine is encouraged by feeding lactose or other carbohydrates. A decrease in dietary protein sometimes prevents putrefaction. Clinical experience has amply confirmed the fact that ordinary intestinal antiseptics are of little value in combating the common intestinal infections,¹ and that adjustment of the diet is a more efficacious procedure. Infants have a limited ability to prevent bacterial invasion of the small intestine, and they show a greater incidence of diarrhea and vomiting.

Many of the symptoms of gastro-intestinal intoxication, previously ascribed to bacterial toxins and aporrhegas, are now recognized as reflex symptoms. The headache, irritability, fatigue, insomnia, and drowsiness formerly attributed to so-called autointoxication are actually associated with constipation and are caused by nervous stimuli from the intestine. The detoxicating mechanisms which the body efficiently employs in disposing of bacterial aporrhegas are outlined on page 430. These chemical detoxications are functions of the intestinal mucosa, the liver and, to some extent, of the general tissues.

¹ However, sulfonamides are efficacious in the treatment of dysentery.

EXAMINATION OF FECES

The fecal bulk is increased by defective intestinal absorption, increased peristalsis, or the excretion of mucus, blood, or pus. The feces become gray in color when steatorrhea is present, particularly in biliary obstruction. During diarrhea, the feces may occasionally be greenish in color because of the presence of biliverdin. Increased bacterial fermentation generally imparts to feces a light brown color, an acid reaction, and many gas bubbles. Putrefactive action results in dark brown feces of foul odor and slightly alkaline reaction. The feces of rachitic patients become more alkaline, and pH values increase throughout the intestinal tract; return to a normal reaction is effected by vitamin D therapy.

During digestive disturbances, the feces often contain abnormal amounts of such food residues as fats, fatty acids, soaps, sterols, muscle fibers, connective tissue shreds, starch, and calcium salts. Microscopic examination of the feces reveals the exogenous food elements, cellular detritus from the intestinal tract, micro-organisms, parasites, blood, and mucus. Blood from the large intestine is easily recognizable by microscopic examination and by its color. Occult fecal blood is not recognizable microscopically, but its detection is important in the diagnosis of carcinoma, ulcers, and other lesions. The benzidine, guaiac, and phenolphthalein tests for hemoglobin are used for this purpose (page 530). Tests for occult blood should be made on feces formed from meat-free diets, since meat contains indigestible chromoprotein which imparts positive tests to normal feces. When human blood is given by mouth, about 90 per cent of the prosthetic radical in its hemoglobin appears in the feces as hematin.

In pathological feces, the neutral fats can be stained with Sudan III; the fatty acids are observed, microscopically, as needle-like crystals. Soaps may also be crystalline, but they are usually amorphous. A marked increase in fecal fat, and the presence of undigested muscle fibers, suggests pancreatic pathology. A great preponderance of fatty acids and soaps indicates biliary obstruction, extensive hepatic disease, sprue, or celiac disease. Fat absorption is hindered by increased intestinal motility, and, in rare cases, by tubercular or cancerous obstruction of the intestinal lymph glands, filariasis with lymph fistulae, and amyloidosis of the intestinal mucosa. The undigested meat fibers of feces show transverse striations and angular ends. Their presence in abnormal quantities, termed *creatorrhea*, indicates either very rapid passage through the intestine, or impaired pancreatic function. Starch grains are stained blue by dilute iodine solutions. Their occurrence in the feces is generally due to improper diet, or to vague digestive disorders other than pancreatic disease. When the pancreatic ducts are occluded, amylase is the last pancreatic enzyme to disappear from the intestinal tract. Digestive enzymes appear in the feces in detectable quantities during diarrhea or increased peristalsis.

In closing this discussion of digestive pathology, the author desires again to direct the attention of the student to the quotation at the beginning of the chapter. The tests and dietary principles which have been outlined provide a nucleus about which basic concepts may be crystallized. Individual variations in the symptoms of gastro-intestinal disease, and the dietary prejudices and preferences of patients present complex problems which require the intelligent application of basic physiological knowledge.

BIBLIOGRAPHY

FUNCTION

General

- BABKIN, B. P. *Secretory Mechanism of the Digestive Glands*. New York, Hoeber, 1944.
- IVY, A. C. Internal secretions of the gastro-intestinal tract. *J. A. M. A.*, 117 : 1013, 1941.

Gastric Juice

- HOLLANDER, F. Hydrochloric acid formation in the stomach. *Gastroenterology*, 1 : 401, 1943.
- WOLF, S., and WOLFF, H. G. *Human Gastric Function*. London, Oxford Univ. Press, 1944.

Pancreatic Juice

- MCCLURE, C. W. The exocrine functions of the pancreas. *Ann. Int. Med.*, 10 : 1848, 1937.

Succus Entericus; Intestinal Mucosa

- FLOREY, H. W., *et al.* The secretions of the intestine. *Physiol. Rev.*, 21 : 36, 1941.

Bile

- SOBOTKA, H. *Physiological Chemistry of Bile*. Baltimore, Wm. Wood, 1937.

Gallbladder

- IVY, A. C. Physiology of the gallbladder. *Physiol. Rev.*, 14 : 1, 1934.

Absorption

- VERZAR, F., and MCDougALL, E. J. *Absorption from the Intestine*. New York, Longmans, Green, 1936.

Large Intestine

- STRASBURGER, J. The intestine as an excretory organ. *Bethe's Handb.*, 4 : 681, 1929.
STRASBURGER, J. The feces. *Bethe's Handb.*, 4 : 696, 1929.

Intestinal Bacteria

- MAGNUS-ALSLEBEN, E. Digestive action of micro-organisms in man. *Bethe's Handb.*, 3 : 1027, 1927.
STEPHENSON, M. Bacterial Metabolism. Ed. 2. New York, Longmans, Green, 1939.
WERKMAN, C. H., and WOOD, H. G. Metabolism of bacteria. *Botan. Rev.*, 8 : 1, 1942.

PATHOLOGY

General

- ALVAREZ, W. C. Introduction to Gastro-Enterology. New York, Hoeber, 1940.
DACK, G. M. Food Poisoning. Chicago, Univ. of Chicago Press, 1943.
PORTIS, S. A. Diseases of the Digestive System. Ed. 2. Philadelphia, Lea and Febiger, 1944.
REHFUSS, M. E. Indigestion, Its Diagnosis and Management. Philadelphia, Saunders, 1942.
WILBUR, D. L. The effects of vitamin deficiency on the gastro-intestinal tract. *Am. J. Digest. Dis. & Nutrition*, 6 : 610, 1939.

Dietary Treatment

- ANDRESEN, A. F. R. Dietary principles in the treatment of gastro-intestinal diseases. *Am. J. Digest. Dis. & Nutrition*, 4 : 1, 1937.
BRIDGES, M. A. Dietetics for the Clinician. Ed. 4. Philadelphia, Lea and Febiger, 1941.
MCLESTER, J. S. Nutrition and Diet in Health and Disease. Ed. 4. Philadelphia, Saunders, 1944.
WOHL, M. G. Dietotherapy. Philadelphia, Saunders, 1945.

Salivary and Dental Pathology

- American Association for the Advancement of Science. Fluorine and Dental Health. Lancaster, Science Press, 1942.
COX, G. J. A critique of the etiology of dental caries. *Vitamins and Hormones*, 2 : 255, 1944.
MEAD, S. V. Diseases of the Mouth. Ed. 5. St. Louis, Mosby, 1940.
SCHOUR, I., and MASSLER, M. The effects of dietary deficiencies on the oral structures. *Physiol. Rev.*, 25 : 442, 1945.

Gastric Pathology

- BOCKUS, H. L. Gastro-enterology. Vol. I. Philadelphia, Saunders, 1943.
EUSTERMANN, G. B., and BALFOUR, D. C. The Stomach and Duodenum. Philadelphia, Saunders, 1935.

Biliary Pathology

- BOYCE, F. F. The Role of the Liver in Surgery. Springfield, Thomas, 1941.
- HORRALL, O. N. Bile, Its Toxicity and Relation to Disease. Chicago, Univ. of Chicago Press, 1938.
- LICHTMANN, S. S. Diseases of the Liver, Gallbladder and Bile Ducts. Philadelphia, Lea and Febiger, 1942.
- MATEER, J. G., *et al.* Liver function tests. *J. A. M. A.*, 121 : 723, 1943.
- WALTERS, W., and SNELL, A. B. Diseases of the Gallbladder and Bile Ducts. Philadelphia, Saunders, 1940.
- WEISS, S. Gallbladder and Bile Ducts. Chicago, Year Book Publishers, 1944.

Intestinal Pathology

- BARGEN, J. A. The Modern Management of Colitis. Springfield, Thomas, 1943.
- BOCKUS, H. L. Gastro-enterology. Vol. II. Philadelphia, Saunders, 1944.
- FELSEN, J. Dysentery, Colitis, and Enteritis. Philadelphia, Saunders, 1945.
- MANSON-BAHR, P. The Dysenteric Disorders. Baltimore, Williams and Wilkins, 1939.
- POTH, E. J. Sulfonamides as therapeutic agents in intestinal antisepsis. *Internat. Abstr. Surg.*, 78 : 373, 1944.
- WANGENSTEEN, O. H. Intestinal Obstructions. Ed. 2. Springfield, Thomas, 1942.

Pancreatic Pathology

- MYERS, V. C. Blood amylase in diseases of the pancreas. *Gastroenterology*, 1 : 617, 1943.
- PORTIS, S. A. Diseases of the Digestive System. Ed. 2. Philadelphia, Lea and Febiger, 1944.
- THOMAS, J., and SCHULTZ, F. W. Pancreatic steatorrhea. *Am. J. Dis. Child.*, 56 : 336, 1938.
- WEISS, S. Diseases of the Liver, Gall Bladder, Ducts, and Pancreas. New York, Hoeber, 1935.

CHAPTER IV

LIPIDES



CHEMISTRY

"In action, as in science, not all that exists is relevant; and neither the fullness of life nor the fullness of knowledge can be attained without scientific organization which ignores or eliminates the irrelevant." — MORRIS R. COHEN

CLASSIFICATION OF LIPIDES

The lipides include true fats and biologically related substances. Lipides are usually insoluble in water but are soluble in the fat solvents, ether, chloroform, benzene, and so forth. However, phospholipides, soaps, bile salts, and saponins form colloidal solutions in water, while the cerebrosides, sphingomyelins, saponins, certain soaps, and glycerol are rather insoluble in ether. A classification of lipides and their chemical units is given in Table 29.

The fat-soluble chemical units of lipides are termed *derived lipides*. *Simple lipides* include the food fats and oils; the *complex lipides* are typical constituents of living protoplasm. The fat-soluble hormones, vitamins, bile acids, aglycones, and hydrocarbons are closely related, both biologically and chemically, to true lipides.

FATTY ACIDS

The characteristic reactive radical of fatty acids is the carboxyl radical (—COOH). In Table 30, fatty acids are classified according to the degree of saturation and the secondary chemical radicals. The distribution of fatty acids in important fats is given in Table 32, page 185. Four saturated fatty acids with which the student should become familiar are: butyric, palmitic, stearic, and lignoceric acids. It is noteworthy that most fatty acids of the natural lipides have normal or straight chains, with even numbers of carbon atoms. Phthioic and tuberculostearic acids, obtained from lipides of the *Mycobacterium tuberculosis*, have branched chains. Phthioic acid and other fatty acids with methyl side chains stimulate proliferation of monocytes, epithelioid and giant cells, and incite tubercle formation. Quartz dust produces a similar silicotic cellular reaction in the lungs with marked increase of tissue phospholipides.

TABLE 29

LIPIDES

CHEMICAL UNITS, OR HYDROLYTIC PRODUCTS

Derived lipides

Fatty acids
Fatty aldehydes
Fatty alcohols

Simple lipides

Fats Fatty acids, glycerol
Waxes Fatty acid, alcohol other than glycerol
Sterol esters Fatty acid, sterol

Complex lipides

Phospholipides Fatty acids, glycerol, phosphoric acid, nitrogen bases
Glycolipides (cerebrosides) Fatty acid, nitrogen base, sugar

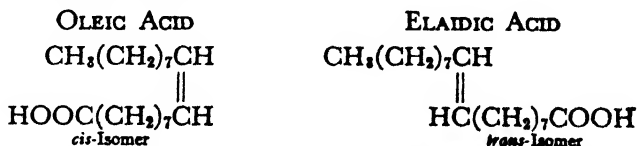
Related substances

Sterides (derivatives of phenanthrene)
Sterols (steride alcohols)
Sterones (steride ketones)
Bile acids (steride hydroxy acids)
Aglycones or genins (steride lactones)
Hydrocarbons and their fat-soluble derivatives
Aliphatic
Carotenoid (lipochromes)
Chroman derivatives
Naphthalene derivatives

All fatty acids are soluble in fats and in fat solvents; and saturated fatty acids having less than five carbon atoms are very soluble in water. The solubility of fatty acids in water diminishes rapidly with increasing molecular weight, and the higher fatty acids are insoluble waxy solids. Saturated fatty acids with less than ten carbon atoms are sharp-smelling liquids which distil with steam.

Unsaturated Fatty Acids

These acids have one or more unsaturated linkages, or double bonds, which allow *geometrical isomerism*.¹ Thus, oleic and elaidic acids are geometrical *cis-trans* isomers:



¹ Isomers are substances having the same empirical formula but different structural formulae. Isomerism of saturated fatty acids is due to branching of carbon chains. In unsaturated fatty acids additional isomerism results from (a) positions of unsaturated linkages, and (b) space direction of the portions of the chain at either side of the double bond. The latter is geometrical isomerism.

TABLE 30

FATTY ACIDS

Iodine
Number¹I. *Saturated acids*

Straight chain

Acetic	CH_3COOH
Butyric	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$
Caproic	$\text{CH}_3(\text{CH}_2)_4\text{COOH}$
Caprylic	$\text{CH}_3(\text{CH}_2)_6\text{COOH}$
Capric	$\text{CH}_3(\text{CH}_2)_8\text{COOH}$
Lauric	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$
Myristic	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$
Palmitic	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
Stearic	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$
Arachidic	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$
Behenic	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$
Lignoceric	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$
Cerotic	$\text{CH}_3(\text{CH}_2)_{24}\text{COOH}$
Montanic	$\text{CH}_3(\text{CH}_2)_{26}\text{COOH}$
Melissic	$\text{CH}_3(\text{CH}_2)_{28}\text{COOH}$

Branched chain

Tuberculostearic .	$\text{CH}_3(\text{CH}_2)_7\text{CH}(\text{CH}_2)_8\text{COOH}$
	CH_3
Phthioic	$\text{CH}_3(\text{CH}_2)_3\text{CH}(\text{CH}_2)_5\text{CH}(\text{CH}_2)_9\text{CHCH}_2\text{COOH}$
	CH_3 CH_3 CH_3

II. *Unsaturated acids*²

One unsaturated linkage

Decenoic	$\text{CH}_3=\text{CH}(\text{CH}_2)_7\text{COOH}$	157
Dodecenoic	$\text{CH}_3\text{CH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	128
Tetradecenoic	$\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	112
Palmitoleic ³	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	100
Oleic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	90
Isooleic	$\text{CH}_3(\text{CH}_2)_6\text{CH}=\text{CH}(\text{CH}_2)_8\text{COOH}$	90
Vaccenic	$\text{CH}_3(\text{CH}_2)_8\text{CH}=\text{CH}(\text{CH}_2)_9\text{COOH}$	90
Gadoleic ⁴	$\text{CH}_3(\text{CH}_2)_9\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	82
Cetoleic	$\text{CH}_3(\text{CH}_2)_9\text{CH}=\text{CH}(\text{CH}_2)_9\text{COOH}$	75
Erucic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}\text{COOH}$	75
Nervonic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{11}\text{COOH}$	69

Two unsaturated linkages

Linoleic	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	181
------------------	--	-----

¹ Several of these iodine numbers are calculated values.² Organic chemists indicate the position of the double bond by the symbol Δ. Thus, oleic acid is Δ⁹:10 acid.³ Also termed hexadecenoic acid, or zoomaric acid.⁴ Jeoleic acid from cod liver oil is a mixture containing some gadoleic acid.

TABLE 30 (Cont.)

FATTY ACIDS

		Iodine Numbers ¹
Three unsaturated linkages		
Linolenic . . .	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	274
Isolinolenic . . .	$\text{C}_{19}\text{H}_{30}\text{O}_2$	274
Jecoric . . .	$\text{C}_{19}\text{H}_{30}\text{O}_2$	274
Four unsaturated linkages		
Arachidonic . . .	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{COOH}$	334
Therapic ; . .	$\text{C}_{18}\text{H}_{32}\text{O}_2$	
Stearidonic . .	$\text{C}_{18}\text{H}_{32}\text{O}_2$	
Five unsaturated linkages		
Clupanodonic . .	$\text{CH}_3\text{CH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}=\text{CH}(\text{CH}_2)_2\text{COOH}$	384
Six unsaturated linkages		
Nisinic ; ; . .	$\text{C}_{24}\text{H}_{40}\text{O}_2$	428

III. Cyclic acids

Hydnocarpic . .	$\begin{array}{c} \text{CH}=\text{CH} \\ \quad \diagup \\ \text{CH}_2-\text{CH}_2 \\ \quad \diagdown \\ \text{CH}=\text{CH} \end{array} \text{CH}(\text{CH}_2)_{10}\text{COOH}$	101
Chaulmoogric . .	$\begin{array}{c} \text{CH}=\text{CH} \\ \quad \diagup \\ \text{CH}_2-\text{CH}_2 \\ \quad \diagdown \\ \text{CH}=\text{CH} \end{array} \text{CH}(\text{CH}_2)_{12}\text{COOH}$	91
Gorlic	$\begin{array}{c} \text{CH}=\text{CH} \\ \quad \diagup \\ \text{CH}_2-\text{CH}_2 \\ \quad \diagdown \\ \text{CH}=\text{CH} \end{array} \text{CH}(\text{CH}_2)_6\text{CH}=\text{CH}(\text{CH}_2)_4\text{COOH}$	181

IV. Oxy acids

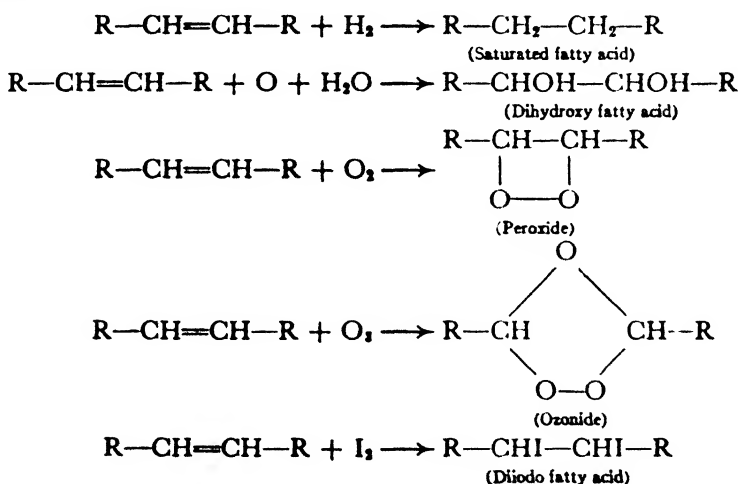
Saturated		
Acetoacetic . .	$\text{CH}_3\text{COCH}_2\text{COOH}$	
β -Hydroxybutyric	$\text{CH}_3\text{CHOHCH}_2\text{COOH}$	
Lanopalmic . .	$\text{C}_{16}\text{H}_{32}\text{O}_4$	
Dihydroxystearic	$\text{CH}_3(\text{CH}_2)_7\text{CHOHCHOH}(\text{CH}_2)_7\text{COOH}$	
Cerebronic . .	$\text{CH}_3(\text{CH}_2)_{21}\text{CHOHCOOH}$	
Lanoceric . . .	$\text{C}_{25}\text{H}_{50}\text{O}_4$	
Mycolic	$\text{C}_{68}\text{H}_{170}\text{O}_4$	
Unsaturated		
Ricinoleic . . .	$\text{CH}_3(\text{CH}_2)_9\text{CHOHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	85
Oxynervonic . .	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{15}\text{CHOHCOOH}$	66

¹ Several of these iodine numbers are calculated values.

Oleic acid can be transformed into elaidic acid by treatment with nitrous acid. Similarly, erucic acid can be converted into its *cis*-isomer, brassidic acid; and ricinoleic acid into its *trans*-isomer, ricinelaidic acid. With increasing numbers of unsaturated linkages, more geometrical isomers are possible. The most abundant unsaturated fatty acids are oleic, nervonic, linoleic, linolenic, and arachidonic acids. Linoleic and linolenic acids have *cis* configurations at the 9 : 10 position. Liver oils contain highly unsaturated fatty acids with fishy odors (Table 32, page 185).

Reactions of Unsaturated Fatty Acids

The unsaturated carbon atoms of fatty acids have characteristic chemical activities, such as the addition of hydrogen, oxygen, ozone, and halogens:



Hydrogenation converts unsaturated fatty acids to the corresponding saturated acids. Lard and butter substitutes are produced, commercially, by partial hydrogenation of unsaturated vegetable oils.

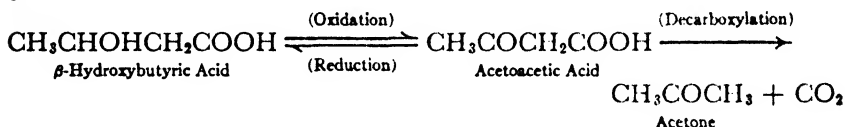
Unsaturated fatty acids are easily oxidized, and those with several unsaturated linkages are highly auto-oxidizable. Drying oils of the paint trade, such as linseed, tung, soybean, and China wood oils, contain highly unsaturated fatty acid units; they thicken in contact with oxygen to form polymerized films. Unsaturated fatty acids having the *cis* configuration at the 9 : 10 position are converted to peroxides by the enzyme, lipoxidase. The reaction of fatty acids with ozone is used to determine the positions of unsaturated linkages. Fatty acid ozonides can be hydrolyzed at the positions of the double bonds; the nature of the resulting aldehyde and acid fragments indicates the chain lengths of the saturated portions of the fatty acid molecule.

The addition of halogens, for example, iodine, to unsaturated linkages of fatty acids and lipides is the basis of an important analytical reaction. The *iodine number*, a measure of the degree of saturation, is defined as the per cent of iodine taken up by a lipide. To determine the iodine number, a quantitative solution of halogen is allowed to react with a weighed amount of lipide in chloroform solution. The excess halogen is subsequently determined by adding potassium iodide and titrating with thio-sulfate. Iodine numbers of natural fatty acids are given in Table 30; those of important fats and oils, in Table 33, page 186; and those of complex lipides, in Table 34, page 188.

Oxy and Cyclic Fatty Acids

The *oxy fatty acids* (Table 30) are characteristically insoluble in petroleum ether. With the exception of acetoacetic acid, they have one or more hydroxyl radicals attached to the carbon chain. Cerebronic acid is a unit of the cerebroside. Mycolic acid, which contains one hydroxyl and one methoxyl radical, is responsible for acid-fastness of *Mycob. tuberculosis*. Ricinoleic acid is the laxative component of castor oil.

The chemical interrelation of the acetone bodies is shown in the diagram:

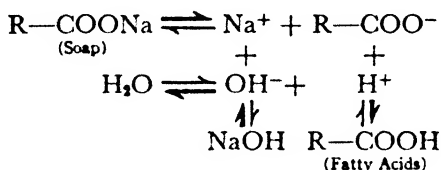


The biological conversion of β -hydroxybutyric acid into acetoacetic acid is controlled by oxidizing-reducing systems, and acetone formation is catalyzed by acetoacetic decarboxylase. *Acetoacetic acid* tends to decompose *in vitro* into acetone and carbon dioxide. Acetoacetic acid is detected in urine by the appearance of a red color on the addition of ferric chloride solution (Gerhardt's test). This reaction is sensitive to 1 mg. per cent of acetoacetic acid in aqueous solution, or 10 mg. per cent in urine. Phenolic drugs (antipyrin, aspirin, salicylates, etc.) give similar colorations, which differ, however, in that they are stable to boiling. The red color given by acetone and acetoacetic acid with salicylaldehyde is the basis of a method for the quantitative estimation of acetone bodies. *β -Hydroxybutyric acid* gives none of the color tests, unless it is first oxidized to acetoacetic acid. *Acetone* is detected by the red colors produced in alkaline solutions of sodium nitroprusside (Lange's test) or salicylaldehyde, or by the reduction of an alkaline mercury-silver reagent (Scott-Wilson reagent). Acetone is a volatile liquid and is, therefore, detectable in the breath of ketosis patients.

The *cyclic fatty acids* are characteristic units of chaulmoogra oil, which is used in the treatment of leprosy.

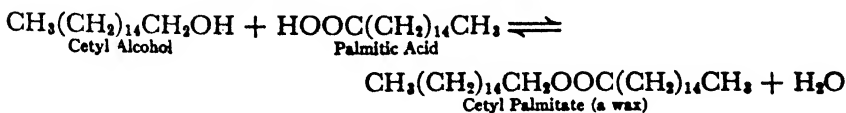
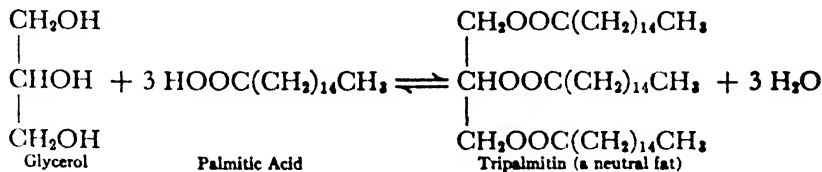
General Reactions of Fatty Acids

Two general radicals in fatty acids are (1) the hydrocarbon chain, which confers lipide characteristics; and (2) the carboxyl radical, which allows the formation of salts, esters, amides, and aldehydes. The fatty acids are feebly dissociated (Table 1, page 4), and aqueous solutions of their salts are alkaline (pH above 8.0). The salts of higher fatty acids are *soaps*. Fatty acids may be determined by titration with sodium alcoholate to the endpoint of phenolphthalein. Aqueous solutions of sodium and potassium soaps are colloidal; but, on sufficient dilution, they approach the status of true solutions. Colloidal soap solutions can be salted out, flocculated, or converted to gels. Soaps cause marked lowering of surface tension. Dilute soap solutions gradually become cloudy, because of the separation of fatty acids produced by salt hydrolysis:



Calcium, magnesium, and heavy metal salts of fatty acids are quite insoluble in water. For this reason, hard water precipitates the household sodium soaps. Lead salts of many unsaturated fatty acids are much more soluble in alcohol and ether than are those of saturated fatty acids.

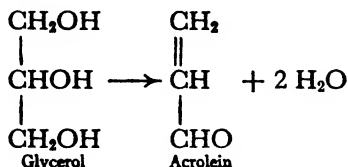
The most important biological reaction of the carboxyl radical is *esterification*, or combination with an alcohol. Simple lipides are esters of fatty acids and alcohols. They are formed by the following reactions:



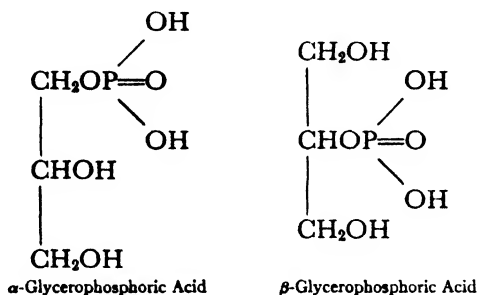
The glycerol unit of phospholipides is partially esterified with fatty acids.

In the cerebrosides and sphingomyelins, the fatty acids are combined with the amino radical of sphingosine to form *amides*:

fate and sulfuric acid, remove two molecules of water from hot glycerol to form the irritating gas, *acrolein*:



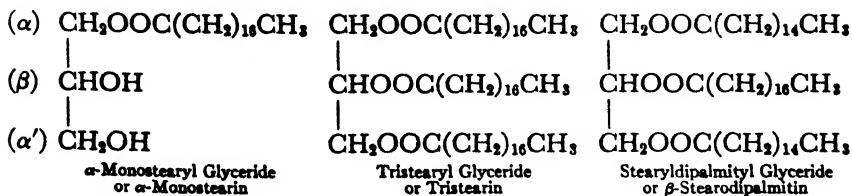
Glycerol is more easily oxidized than the fatty alcohols; it is esterified with fatty acids and with phosphoric acid to form fats and *glycerophosphoric acids*, respectively. There are two isomeric glyceromonophosphoric acids:



The naturally occurring α -glycerophosphoric acid is the *l*-isomer. In the lecithins and cephalins, glycerol is esterified with both fatty acid and phosphoric acid.

FATS

Fats are glycerides or glycerol esters of fatty acids. Esterification of all the alcohol radicals of glycerol produces a triglyceride, or *neutral fat*, the typical constituent of animal fats and vegetable oils. The average neutral fat consists of approximately 90 per cent fatty acids and 10 per cent glycerol, by weight. Usually, the three fatty acid units of natural fats are not identical; such fats are termed *mixed glycerides*. The nomenclature of glycerides is illustrated by the following examples:

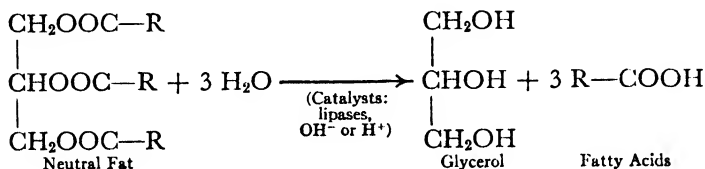


X-ray studies show that the fatty acid radical at the β carbon atom of a triglyceride is *trans* to the two other fatty acid radicals ("tuning fork")

structure). The melting point of a mixed glyceride depends on the nature and arrangement of the component fatty acids. The fatty acid distributions of Table 32 indicate that complex mixtures of neutral fats are present in natural fats and oils. Since short-chain and unsaturated units preponderate in oils, the latter have low melting points. Fats are excellent solvents for other lipides, and are closely associated with them in biological materials.

Reactions of Fats

Fats are hydrolyzed by alkalis or by lipases (less readily, by acids), as shown in the following equation:



Hydrolysis by alkali is called *saponification*, because the liberated fatty acids combine with the alkali to form soaps. Monovalent soaps aid fat hydrolysis by emulsifying the unhydrolyzed fat. Other good emulsifying agents for fats are: bile salts, lecithins, saponins, proteins, and acacia. Steapsin readily hydrolyzes neutral fats and phospholipides, while esterases catalyze the hydrolysis of simple esters of fatty acids, such as ethyl butyrate.

Fats slowly undergo spontaneous hydrolysis and oxidation, and so become rancid in the presence of moisture, light, and oxygen. The oxidative reactions involved are those given on page 179. Since the oxidation occurs chiefly at unsaturated linkages, the iodine number falls as rancidity develops. Free fatty acids catalyze this oxidation, but bacteria cause even more rapid and extensive rancidity. Certain phenols are powerful inhibitors of fat auto-oxidation; these are termed *antioxygens*, *antioxidants*, or *inhibitors*. Oxidative rancidity is detected by color reactions involving the action of the organic peroxides formed. In the Kreis test for rancidity, phloroglucinol is conjugated with some of these oxidation products to form red compounds.

Fats are characterized by their solidification points, iodine numbers, and saponification, Reichert-Meissl and acetyl values (Table 33). The iodine number has been discussed on page 180. The *saponification value* is the number of milligrams of potassium hydroxide neutralized by fatty acids during the alkaline hydrolysis of 1 gm. of fat. It varies with the average molecular weight of the fatty acid units. Butter and coconut oil contain larger proportions of short-chain acids than other common fats do (Table 32) and, therefore, have higher saponification values. The *Reichert-Meissl value* is the ml. of 0.1 N alkali equivalent to the volatile

TABLE 32

APPROXIMATE COMPOSITION OF FATS¹

(Per Cent of Total Fatty Acids)

FAT OR OIL	La	M	P	S	A	Lg	O	L	Ln	OTHER FATTY ACIDS
Beef	t	3	27	23	1		36	2	t	Arachidonic t, palmitoleic 2, tetradecenoic t, vaccenic 4
Beeswax			p							Cerotic p, melissic p, montanic p
Bone marrow . .		3	32	15			43	3		Palmitoleic 3
Butter	3	10	25	10	t		32	4		Arachidonic t, butyric 3, capric 2, caproic 2, caprylic 1, cerotic t, decenoic t, dodecenoic t, palmitoleic 4, tetradecenoic 1
Castor			t	t			8	3		Dihydroxystearic 2, ricinoleic 83
Chaulmoogra . .	p		4				15	p		Chaulmoogric 22, gorlic 23, hyd-nocarpic 35
Chicken		t	25	5			40	20		Palmitoleic 7
Cocconut	45	18	8	2	t		7	2		Capric 7, caproic t, caprylic 8, palmitoleic 1
Cod liver		4	10	1				p	p	Arachidonic p, capric p, clupanodonic 10, erucic p, gadoleic p, jecoleic 20, jecoric 17, nisinic p, palmitoleic 6, stearidonic p, therapeutic 20
Corn			7	3	t	t	45	40		
Cottonseed . . .		t	20	2	t		30	46		Palmitoleic p
Egg		1	25	5	t		45	17	3	Palmitoleic p, clupanodonic p
Human	t	4	25	7			45	10		Arachidonic t, palmitoleic 6, tetradecenoic t
Lard	t	3	25	13	t		47	10		Palmitoleic 2, tetradecenoic t, vaccenic p
Lard substitute ²			{...33...}				45	8		Isooleic 12
Linseed	t	6	4		t		9	35	40	Isolinolenic 3
Milk, human . .	6	0	23	9			34	7		Capric 2
Mutton			25	30			35	4		Vaccenic 2
Oat			10				60	30		
Olive	t	8	2	t			82	5		Palmitoleic 1
Peanut	t	t	7	4	3	3	60	20		Behenic t, caprylic t
Pecan		t	3	2	t		80	15		
Rape seed . . .		1	t	2	t	2	20	15	2	Behenic t, erucic 50
Rice		t	12	2	t	t	45	35		
Salmon		4	13	1			25			Arachidonic p, palmitoleic 10, tetradecenoic t
Soya bean		t	9	4	1	t	30	50	3	Palmitoleic t
Wool	t	t	p	p			t			Cerotic p, lanoceric p, lanopalmitic p

A-Arachidic acid
L-Linoleic acid
La-Lauric acid
Lg-Lignoceric acid

Ln-Linolenic acid
M-Myristic acid
O-Oleic acid

P-Palmitic acid
p-Prent
S-Stearic acid
t-Trace

¹ For variation in the composition of animal fats, see page 218.² Hydrogenated vegetable oils.

TABLE 33

APPROXIMATE ANALYTICAL CONSTANTS OF FATS

FAT OR OIL	REICHERT-MEISSL VALUE	IODINE NUMBER	SOLIDIFICATION POINT	ACETYL VALUE
Beef	0.5	40	34	9 ¹ V
Beeswax	0.4	8	61	15
Bone marrow	2.0	45 V	30	
Butter	25.0	30	21	5 ¹ V
Castor	1.5	85	— 15	150 ¹
Cat	0.9	55	26	
Chaulmoogra		100	23	
Chicken	1.0	65 V	24	45
Cocoanut	7.0	10	18	2 ¹
Cod liver	0.2	150 V	— 3	1 ¹
Corn	4.0	120 V	— 15	16 ¹
Cottonseed	1.0	110	V	15 ¹
Dog	0.5	60 V	25	
Egg	0.5	75 V	9	
Goose	0.5	60	22	0.5
Hair	2.3	65	23	
Human, adult	0.3	70 V	15	
Human, child	2.5	50 V		
Lard (fat)	0.2	55 V	28	3 ¹
Lard (oil)	0.1	70 V	2	3 ¹
Linseed	1.0	190 V	— 24	4 ¹
Milk, human	2.0	45		
Mutton	0.3	40 V	38	
Oat	0.6	110	12 V	
Olive	1.0	85	— 2	10 ¹
Peanut	0.5	90	3	6
Pecan	0.1	100		7
Rabbit	2.1	85 V	23	
Rape seed	0.5	100	— 10	15
Rice	1.0	100		
Salmon	0.6	160		
Sardine	1.7	150 V	21	22
Soya bean	1.5	130	— 13	5
Wheat	0.5	120	0	
Wool	8.0	25	39	23

¹ Corrected for volatile fatty acids, which distil with the acetic acid.

V — Very variable.

fatty acid from 5 gm. of hydrolyzed fat. It is a direct measure of the content of short-chain fatty acid units. The *acetyl value* is the number of milligrams of potassium hydroxide equivalent to the acetic acid liberated

by hydrolysis of 1 gm. of acetylated fat. It is a measure of the hydroxy acid content. The acetyl value of castor oil is very high (Table 33).

The quantitative determination of lipides in blood or tissues requires extraction with alcohol-ether mixtures, and saponification by sodium ethylate. The unsaponifiable matter (sterols and fatty alcohols) is separated by ether extraction of the alkaline hydrolyzate. The fatty acids are liberated from their soap solution by the addition of acid, and are then extracted with a fat solvent, such as petroleum ether. In Bloor's method, the fatty acids are oxidized with standard potassium dichromate and silver dichromate, in sulfuric acid solution; the excess dichromate is then determined by iodometric titration.

WAXES

The waxes are esters of fatty acids and alcohols of high molecular weight. The sterol esters, which are usually included in this classification, will be considered later. The solid plant and bacterial waxes have received most study; liquid waxes and sterol esters are found in many animal tissues. Wool fat, beeswax, and sperm oil contain large proportions of waxes. The fatty acid units of wool fat (lanolin) and of beeswax are given in Table 32. Sterol units (cholesterol, agnosterol, and lanosterol) and carnaubyl and ceryl alcohols are also present. The fatty alcohol units of beeswax are carnaubyl, ceryl, and melissyl alcohols (Table 31, page 182). Beeswax contains several solid hydrocarbons (page 205). The alcohol of tubercle bacillus wax is a sugar (trehalose); a methoxyl fatty alcohol, phthiocerol ($C_{34}H_{67}(OH)_2OCH_3$), is also present. The waxes are not hydrolyzed by ordinary lipases. They are saponified slowly by alkali.

PHOSPHOLIPIDES

These complex dextrorotatory lipides are present in all cells. Especially large concentrations are found in the myelinated portions of the nervous system. Phospholipides are insoluble in acetone, which is, therefore, used to separate them from lipid mixtures. In living cells, they exist largely as phospholipide-protein complexes. Such complexes are not stained by Sudan III, and they are with difficulty soluble in ether unless alcohol is also present. When proteins are flocculated from biological mixtures, a part of the phospholipide is removed. Phospholipides are excellent emulsifying agents for fats. In water, the phospholipides form gels, liquid crystals, and *myelin forms* (hydrated pseudopod-like processes). The myelin forms display certain movements resembling those of living cells. The phospholipides are feeble buffers; like the proteins, they form dipolar ions (page 358).

The phospholipides of bacteria, which are immunologically important, contain polysaccharides in place of glycerol, and some have peculiar fatty acids (see phthioic acid, page 175). Polysaccharides are also present

in the complex lipides of plants, and in the beef heart phospholipide used as "lipide antigen" for the Wassermann reaction. The principal phospholipides of animal tissues are divided into three groups: lecithins, cephalins, and sphingomyelins.

Lecithins

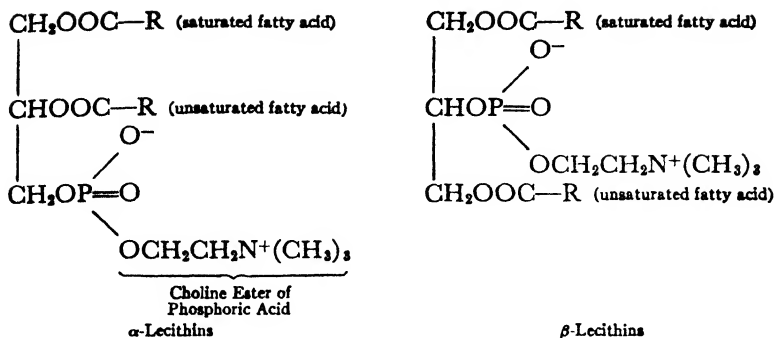
Lecithins are hygroscopic substances; in the presence of oxygen, they change quickly from white waxy solids to brown mixtures because of auto-oxidation of the unsaturated fatty acid units. They form aqueous colloidal solutions which are reversibly flocculated by divalent cations. Lecithins

TABLE 34

IODINE NUMBERS OF COMPLEX LIPIDES AND STEROLS

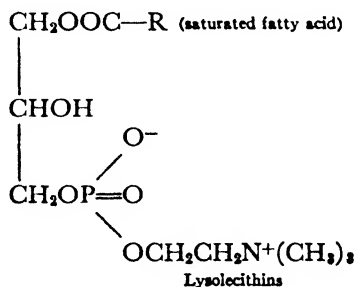
<i>Lecithins</i>	IODINE NUMBER
Adipose tissue	65
Brain	61-105
Egg yolk	47- 70
Kidney	95-115
Liver	72-145
Lysolecithins	0
Muscle	82-140
<i>Cephalins</i>	40- 78
Lysocephalins	0
Phosphatidylethanolamine	78
Phosphatidylserine	33
<i>Sphingomyelin (mixed)</i>	34
Cerebronyl sphingosine	38
<i>Cerebrosides</i>	
Kerasin	31
Nervon	63
Oxynervon	61.5
Phrenosin	31
Psychosin	55
<i>Sterols</i>	
Cholesterol	66
Ergosterol	199
Sitosterol	66

may be regarded as derivatives of neutral fats, in which one fatty acid unit is replaced by the choline ester of phosphoric acid. Their constitution is indicated by the following type formulae:



The term lecithin is frequently applied to a mixture of various lecithins. Liver lecithins consist of about equal proportions of α - and β -lecithins; the former preponderates in the brain, and the latter in egg yolk. Usually, one of the fatty acid units is either palmitic or stearic acid, and the other is oleic, linoleic, linolenic, arachidonic, or clupanodonic acid. However, certain lecithins contain only saturated or unsaturated fatty acid units; and the *plasmalogens*, or acetal phospholipides (small quantities of which are present in tissues), contain palmitaldehyde and stearylaldehyde in place of fatty acids. These aldehydes can be detected by the Feulgen plasmal reaction mentioned on page 182. The iodine number of the mixed fatty acids obtained from lecithins is usually above 100 (Tables 30, page 177, and 34). Liver lecithins are highly unsaturated.

The fatty acids are easily removed from phospholipides by alkali, acid, lecithinases, or lipases. The lecithinases of cobra and bee venoms remove only the unsaturated fatty acid; they are, therefore, specific lecithinases which form *lysolecithins* from lecithins.

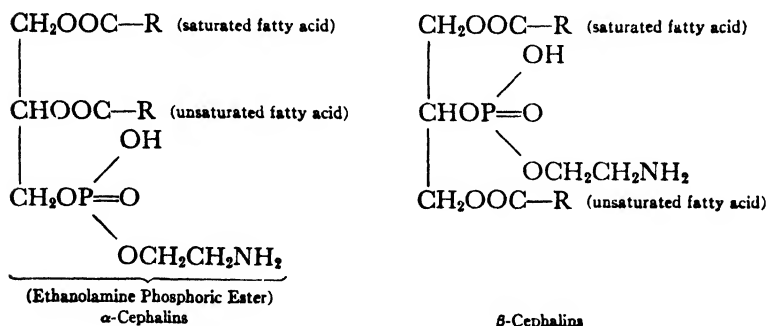


Lysolecithins are immunologically important. They act as powerful hemolytic agents (page 58). They can combine with one molecule of cholesterol to form substances which are not hemolytic. The toxin of type A *Cl. welchii* exerts a different type of lecithinase activity, since it hydrolyzes lecithin to phosphocholine.

The phosphoric acid of lecithins is split from its α - or β -glycerophosphate linkage by the tissue phosphatases. Acids readily hydrolyze choline (Table 35) from lecithins, leaving diglyceride phosphoric or *phosphatidic acids*. Choline is a strong base, which can be determined by precipitating the reineckate and estimating the chromium in the latter colorimetrically. When injected parenterally, choline lowers the blood pressure; but *acetylcholine* is approximately 100,000 times more active in this respect. Acetylcholine is an important hormone produced at parasympathetic or cholinergic nerve endings. Other nitrogenous compounds related to choline are given in Table 35. *Ethanolamine* is a nitrogenous unit of the cephalins. *Sphingosine* is a nitrogenous unit of the sphingomyelins and cerebroside. *Neurine* is an aporrhegma produced by the bacterial putrefaction of choline. Betaine and trimethylamine are found free in tissues and urine. (See also page 367.)

Cephalins

These phospholipides resemble the lecithins; but some cephalin fractions are insoluble in alcohol, and others are more highly dissociated acids, and combine readily with cations. They are hydrolyzed by the lecithinase of cobra venom to *lysocephalins*, which resemble the lysolecithins. The type formulae of the phosphatidylethanolamine cephalins are similar to those of the lecithins, with ethanolamine substituted for choline:



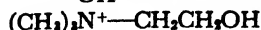
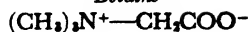
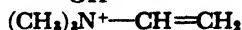
Both α - and β -cephalins are found in tissues. The fatty acid units of these cephalins are stearic, oleic, linoleic, and arachidonic acids. Specific relations of the cephalins to blood clotting have been considered on page 68. Ethanolamine phosphoric ester has been detected in the small intestine and in malignant tumors.

Ordinary cephalin preparations have been shown to be mixtures of (1) *phosphatidylethanolamine*, described above; (2) *phosphatidylserine*, in which ethanolamine is replaced by the amino acid, serine



TABLE 35

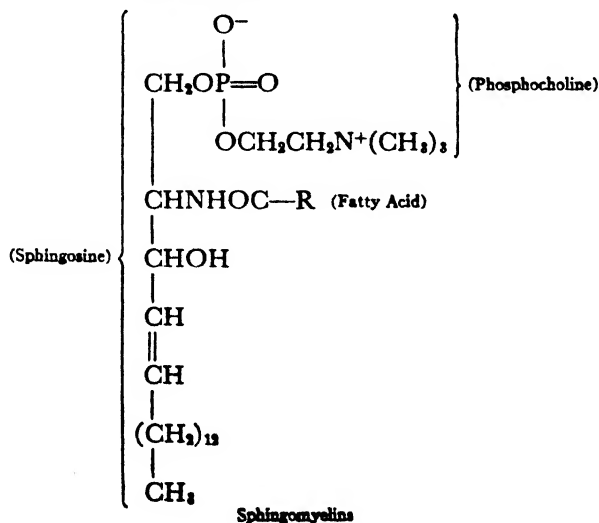
AMINO ALCOHOLS AND DERIVATIVES

Ethanolamine (Aminoethyl Alcohol)*Choline**Acetylcholine**Betaine**Neurine**Trimethylamine**Sphingosine*

and (3) *lipositol*, which contains inositol, *d*-galactose, oleic acid, palmitic acid, stearic acid, cerebronic acid, phosphoric acid, and ethanolamine tartrate units. Brain cephalin contains more phosphatidylserine than do the cephalins of other organs. Phosphatidylethanolamine, like lecithins and sphingomyelins, does not unite readily with cations, and it is rather soluble in alcohol, whereas phosphatidylserine and lipositol combine with cations and are insoluble in alcohol.

Sphingomyelins

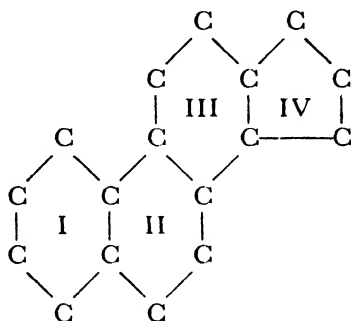
The sphingomyelins differ considerably from the lecithins and cephalins. They are much more stable to oxygen, because of the relative saturation of their fatty acid units (stearic, lignoceric, and nervonic acids). They resemble the cerebroside in their insolubility in ether and their solubility in pyridine. The sphingomyelin type formula is:



ganglioside of beef spleen has lignoceric acid, behenic acid, *d*-galactose and *d*-glucose units. Neuraminic acid is an amino acid which gives a red coloration with orcinol reagent (page 277). The gangliosides are insoluble in ether and acetone, and they form colloidal solutions in water.

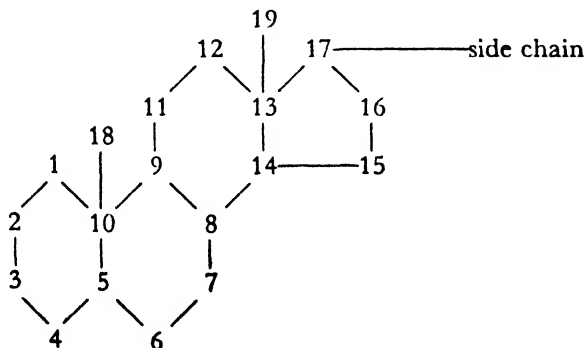
STERIDES

The sterides are derivatives of phenanthrene or, more specifically, of perhydrocyclopentenophenanthrene. The cyclic hydrocarbon skeleton of the latter is:



Each carbon atom of this skeleton is saturated with hydrogen. Pairs of adjoining rings can exhibit geometrical (*cis-trans*) isomerism; but rings II, III, and IV are found to be uniformly *trans* to each other in the natural sterides. The linkage between rings I and II can be either *cis* or *trans*; and the *cis* and *trans* designations in Table 36 refer to this isomerism. Estrogenic hormones, cardiac aglycones, bile acids, and coprosterol belong to the *cis* series, while male sex hormones, phytosterols, cholesterol, and vitamin D are *trans* (or allo-) sterides. The adrenal cortical hormones can be reduced to either *trans* or *cis* sterides.

The carbons of the steride skeleton are numbered as follows:



⁴ The adrenal cortical hormones can be reduced to both pregnane and allopregnane derivatives and are therefore related to both series.

TABLE 36 (Cont.)

STERIDES¹

	DOUBLE BONDS	—OH RADICALS	OTHER RADICALS
Bufotalin (steride unit of bufotoxin ²)	20=21, 22=23	3, 14	=O at 24, (21)—O—(24), —OCOCH ₃ at 7
<i>Cholestane (trans),</i>			
$ \begin{array}{ccccccc} & 20 & 22 & 23 & 24 & 25 & 27 \\ C_{27}H_{48}, & 17-CH-CH_2CH_2CH_2CH-CH_3 \\ & & & & & & \\ & CH_221 & & & & CH_226 & \end{array} $			
Cholestenone	4=5		=O at 3
Dihydrocholesterol, or cholestanol (animal tissues)		3	
Cholesterol (animal tissues)	5=6	3	
Oxycholesterol (animal tissues)	5=6	3, 4	
Dehydrocholesterol	5=6, 7=8	3	
Vitamin D ₃ (liver oils)	5=6, 7=8, 10=18	3	Ring open at 9, 10
Digitogenin (aglycone of digitonin)		2, 3, 6	(16)—O—(22), (22)—O—(26)
<i>Coprostone (cis), C₂₇H₄₈,</i>			
$ \begin{array}{ccccccc} & 20 & 22 & 23 & 24 & 25 & 27 \\ & 17-CH-CH_2CH_2CH_2CH-CH_3 \\ & & & & & & \\ & CH_221 & & & & CH_226 & \end{array} $			
Coprosterol (feces, bacteria)		3	
Coprostenol ³	4=5	3	
Coprostenone (sclerotic aortae)	4=5		=O at 3
Coprostanone			=O at 3
<i>Ergostane (trans), C₂₈H₄₈,</i>			
$ \begin{array}{ccccccc} & 20 & 22 & 23 & 24 & 26 & 28 \\ & 17-CH-CH_2CH_2CH-CH-CH_3 \\ & & & & & & \\ & CH_221 & & 25CH_2 & & CH_227 & \end{array} $			
Dihydroergosterol (ergot, yeast, fungi, etc.)	8=14, 22=23	3	
Ergosterol (ergot, yeast, fungi, etc.)	5=6, 7=8, 22=23	3	
Calciferol (vitamin D ₂)	5=6, 7=8, 10=18, 22=23	3	Ring open at 9, 10
<i>Sitostane or stigmastane (trans), C₂₉H₅₀,</i>			
$ \begin{array}{ccccccc} & 20 & 22 & 23 & 24 & 27 & 29 \\ & 17-CH-CH_2CH_2CH-CH-CH_3 \\ & & & & & & \\ & CH_221 & & 25CH_2 & & CH_228 & \\ & & & & & & \\ & & & 26CH_2 & & & \end{array} $			
Sitosterols (plant oils)	5=6		Position varies
Stigmasterol (plant oils)	5=6, 22=23	3	

¹ See Table 106, page 692, for formulae of steride hormones.² Bufotoxin is Bufotalin —3—O—CO(CH₂)₈CONHCH(CH₂)₈NHC=NH

$$\begin{array}{c}
 | \\
 COOH \quad NH_2 \\
 \text{(Suberylarginine)}
 \end{array}$$
³ "Allocholesterol" is a coordination compound of cholesterol and coprostenol.

Carbons 18 and 19 represent CH_3 radicals attached to the skeleton, as indicated. In Table 36, the important biological sterides are classified as derivatives of this steride skeleton. Inspection of the table reveals that the biological properties of the natural sterides are related to the side chains at carbon 17. The sterides with the shortest side chains are sex hormones, followed by the corpus luteum and cortical hormones, the cardiac aglycones, the bile acids, the ordinary animal sterols, and finally by the plant phytosterols which have the longest side chains. The sterides are optically active.

Sterols

The sterols are hydroxy sterides, and are alcoholic or phenolic in character, depending on the number of unsaturated linkages in ring I. They are soluble in fats and in fat solvents, but are insoluble in water. Since there is a marked tendency for the hydroxyl radical to appear at carbon 3 (Table 36), ring I is most susceptible to oxidation. The hydroxyl radical at carbon 3 can be either *cis* or *trans* to the 18 methyl radical. This gives rise to geometrical sterol isomers, namely, the *natural*, *ordinary*, β , or *cis* forms, and the *epi*-, α , or *trans*-forms. The natural sterols are precipitated by digitonin (page 200), while episterols and such episterides as the bile acids, cardiac aglycones, and androsterone are not precipitated. Neither are the waxes and sterol esters precipitated by digitonin.

The sterols are antagonistic to phospholipides in colloidal and immune reactions (pages 53, 58 and 466). Sterols are usually absent from bacteria, but are widely distributed in plant and animal cells where they exist partly as free sterols and partly as esters of fatty acids (waxes). About 45 per cent of liver oil sterols, 30 per cent of olive oil sterols, and 10 per cent of human fat sterols exist as esters. The sterols of butter, tallow, lard, human erythrocytes, and bile are all in the free form. Tissues contain esterases which hydrolyze sterol esters. The esterases of the liver and pancreas are of particular clinical interest (page 253).

Phytosterols

The phytosterols have long side chains; *sitosterols* and *stigmasterol* occur, in varying proportions, in many plant oils. They usually constitute only 0.2 to 0.6 per cent of the oil, but legume and cereal oils contain as much as 5 per cent. Sitosterol has also been found in toad venom. *Ergosterol* is of particular interest because it is the provitamin or precursor of vitamin D_2 . It is converted into vitamin D_2 by the action of ultraviolet light (280 to 305 $\text{m}\mu$). Ergosterol is the typical sterol of fungi and yeasts, and it is also found in plant oils. In such animal tissues as skin, brain, spinal cord, blood, milk, eggs, and gallstones, ergosterol constitutes approximately 0.5 per cent of the total sterols. Such small quantities are detected by ultraviolet absorption spectra. Because of its marked unsaturation, ergosterol has a high iodine number (Table 34, page 188).

There are numerous other phytosterols which have not been included in Table 36. Agnosterol, with two double bonds, and lanosterol, with three double bonds, are phytosterol-like alcohols of wool fat. These substances, and the cryptosterol of yeast, have thirty carbon atoms. They are not true sterides; since they contain a *picene* skeleton of five benzene rings, they belong to the class of triterpenes and are related to the acids of the plant resins.

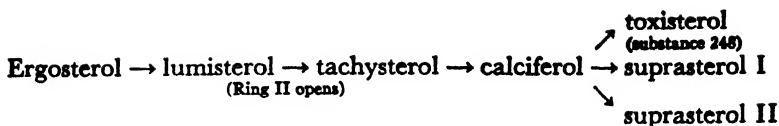
Zoosterols

Cholesterol, a derivative of *trans* cholestane, is the chief protoplasmic sterol of vertebrates. It is a major constituent of nerve tissue, the adrenals and parenchymatous organs. (For distribution, see Table 42, page 238.) Cholesterol is antagonistic to lecithin, particularly in hemolytic systems. This property is not shared by the cholesteryl esters; in fact, the latter act more like the phospholipides. The cholesterol of animal tissues is usually mixed with from 1 to 3 per cent of *dihydrocholesterol* and, at times, with small amounts of *oxycholesterol* and *7-dehydrocholesterol* (Table 36). The latter is not precipitated by digitonin. *Coprosterol* is an isomer of *dihydrocholesterol*. It is derived from coprostane (*cis*). Coprosterol occurs in intestinal contents and feces as a reduction product of unabsorbed sterols.

Color Reactions of Sterols. Unsaturated sterols give Salkowski's and Liebermann's reactions. In the former, a red to purple color appears when a chloroform solution of the sterol is treated with an equal volume of concentrated sulfuric acid. The Liebermann-Burchard reaction is the basis of a method for the quantitative determination of total sterols (both free and esterified), which are usually reported as cholesterol. The substance is dissolved in chloroform and mixed with acetic anhydride and sulfuric acid. The resulting green color is compared, in a colorimeter, with that of a similarly treated cholesterol standard. The esterified cholesterol gives approximately 25 per cent more color than free cholesterol. Glacial acetic acid solutions of such highly unsaturated sterols as ergosterol give a green color on the addition of a dilute chloroform solution of bromine. A color reaction with pyrogallol and aluminum chloride has been used for the determination of calciferol, or vitamin D₂, but the biological assay is more accurate.

Vitamins of the D Series

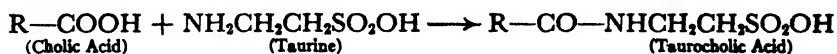
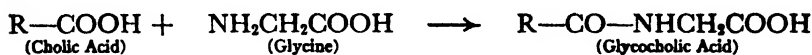
Irradiation, with ultraviolet light, of 5=6, 7=8 unsaturated sterols, or *provitamins*, produces vitamins of the D series. These steride derivatives are tricyclic compounds with ring II open between carbons 9 and 10. The biological activity varies with the different side chains. Vitamin D₂, termed *calciferol*, is made by the irradiation of ergosterol (Table 36, page 195). The photochemical reaction proceeds as follows:



This vitamin gives a characteristic green coloration with acetyl chloride and glycerol dichlorohydrin. Calciferol was first prepared as a coordination compound of lumisterol, and this compound was named vitamin D_1 . Vitamin D_2 is the vitamin of irradiated foods and of viosterol preparations. The characteristic vitamin of fish liver oils and of the skin is vitamin D_3 , produced by the irradiation of dehydrocholesterol (Table 36). Vitamins D_2 and D_3 have approximately equal potencies in rats, while D_3 is somewhat more efficacious in children and is especially active in chickens. Vitamin D_4 is the irradiation product of 22-dihydroergosterol. These substances are relatively stable to oxidation. They are considered in detail on page 668. The *international vitamin D unit* is equal to 0.025 γ of calciferol.

Bile Acids

The bile acids have shorter side chains than do the sterols, and they are characterized by a carboxyl radical at the end of the side chain. The structures of three human bile acids are given in Table 36, page 194. They differ only in the number and location of the hydroxyl radicals. In normal human bile, the bile acids exist chiefly as the salts of conjugated forms. If $R\text{---COOH}$ is used to represent free bile acid, the conjugated acids are formed as follows:



This type of linkage with amino acids is called peptide linkage. The conjugated bile acids are more soluble in water than are the free bile acids. Tauroacids are more highly ionized and less stable than the glycoacids (Table 1, page 4).

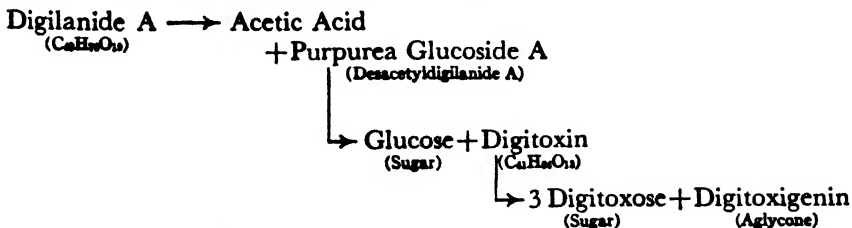
Human bile salts consist of approximately 80 per cent glycocholates and 20 per cent taurocholates. Only 60 per cent are derivatives of *cholic acid*; conjugates of *desoxycholic* and *chenodesoxycholic acids* each constitute 20 per cent of the mixture. Because of its bile salt content, bile can dissolve from 4 to 5 per cent of oleic acid, or 15 per cent of an equal mixture of oleic and stearic acids. Definite *choleic acid* compounds of desoxycholic acid and fatty acids have been crystallized; similar compounds of the conjugated bile acids and fatty acids are known only in solution. The latter seem to be more stable in slightly acid solutions and are, therefore, better adapted to the actual conditions during absorption in the small intestine.

An outstanding property of bile salts is a great surface activity which resembles that of soaps and saponins. This property is important for the biological liberation of adsorbed substances, and for the diffusion of insoluble metabolites through membranes (pages 56 and 149). Bile salts can be detected in biological fluids by methods which show the lowered surface tension. The Hay test for bile salts in urine depends on the flotation of "flowers of sulfur." If the sulfur particles do not float, the urine contains the equivalent of 0.05 per cent or more of glycocholic acid. In the Pettenkofer test for bile acids, a purple color is produced by treating solutions of these substances with concentrated sulfuric acid and a ketose sugar (usually sucrose). The reaction has been made the basis of a method for the quantitative determination of bile acids; but the reaction is not specific and is not given by desoxycholic acid.

Bile acids form water-soluble choleic acids, or coordination compounds with fatty acids, aromatic acids, unsaturated hydrocarbons, certain alcohols and esters, sterols, and so forth. These are molecular compounds formed through secondary valences. Palmitic and higher fatty acids combine with eight molecules of bile acid; myristic, lauric, and capric acids with six molecules; and butyric and caprylic acids combine with four molecules. The tendency to form coordination compounds is also an important property of the saponin sterides, and it is feebly exhibited even by the sterols. (Cf. cholesterol digitonide, allocholesterol, vitamin D₁, etc.)

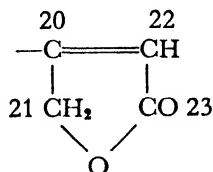
Aglycones

These sterides exist, in plant tissues, as glycosides in which they are united with from one to four molecules of various sugars. Glycoside formation is a common mechanism of plants by which water-soluble substances are produced. The *cardiac glycosides* are present in the seeds and leaves of certain plants. They include various arrow poisons and the digitalis glycosides. The steride units, aglycones or *genins*, of these glycosides constitute the active components of such important cardiac drugs as digitoxin, strophanthin, and ouabain. *Digitoxigenin*, the aglycone of digitalis, is related to the original plant glycoside as follows:



One of the digitoxose units is attached to carbon 3 of the aglycone. In Table 36, page 194, digitoxigenin is shown as a steride whose chain length is intermediate between those of the bile acids and the sex hormones. The

digitoxigenin side chain is an unsaturated lactone, and it is this peculiar ring which endows the cardiac aglycones with their therapeutic properties:



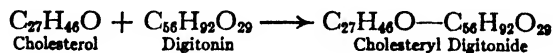
The sugar portion of the cardiac glycoside merely modifies the cardiotonic effect by increasing solubility and diffusion. The cardiac aglycones give a red color with sodium nitroprusside and alkali (the Legal test), but they are usually determined by biological assay. They are not precipitated by digitonin. In small doses, the cardiac glycosides have an emetic action and exert a powerful specific action on heart muscle; they stimulate decompensated heart muscle to greater contractional activity. Cardiac glycosides tend to inhibit phosphatase. Like adrenal cortical hormones, they can protect against lethal doses of potassium salt and have some anti-insulin activity.

Toad Poisons

Cardiac poisons with digitalis-like action are secreted by the skins of certain toads. The cardiotonic portions of these substances are steride genins called *bufagins*; they are united with suberylarginine to form the *bufotoxins*, or toad poisons. The steride from the bufotoxin of the common toad is called bufotalin. Its structure and relations to bufotoxin are shown in Table 36, page 195. Note that it is a lactone of the cholane series, with the same chain length as the bile acids. The suberylarginine unit of bufotoxin corresponds to the sugars of the cardiac glycosides; the cardiotonic action is due to the unsaturated lactone ring in the side chain.

Saponins

The saponins are plant glycosides which contain steride aglycones. They have no cardiac action, but resemble soaps and bile salts in that they lower surface tension tremendously and are very hemolytic (page 58). Saponins are toxic when injected, but not when taken orally. Like the bile acids, saponins unite readily with natural sterols which have a *cis* hydroxyl radical on carbon 3, to form coordination compounds, as, for example, cholesteryl digitonide.



This *digitonide* is insoluble in water and ether; it allows the separation of free cholesterol from its esters in quantitative analysis. By forming similar

compounds, the sterols can detoxify such hemolysins as saponins and lyso-lecithins. Sterol esters do not form digitonides.

The steride aglycone, or *sapogenin*, of digitonin is *digitogenin*. Note that two rings are present in the side chain (Table 36, page 195). In digitonin, the sapogenin is united with four molecules of galactose and one of xylose. Not all plant sapogenins are sterides; some are derivatives of picene with six hydrocarbon rings.

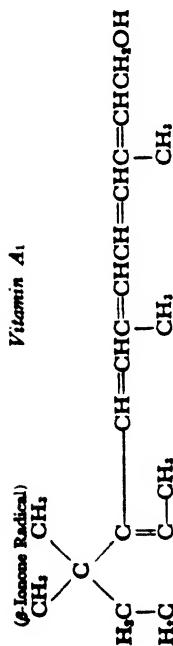
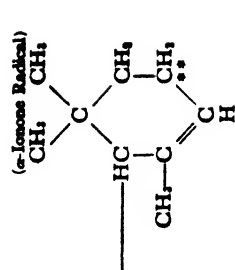
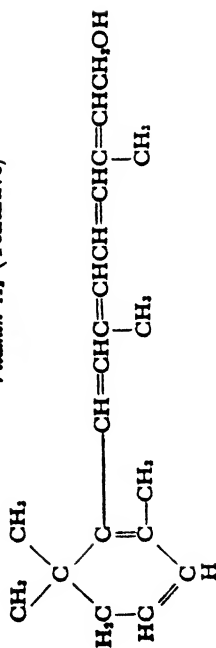
Sex Hormones

These sterides are hydroxy and keto derivatives, or sterols and *sterones*, in which the side chains are either absent or very short. They are divided into three groups: (1) the female sex hormones, highly unsaturated derivatives of estrane (*cis*) which has neither the 18 methyl radical nor the side chain; (2) the male sex hormones, more saturated derivatives of androstane (*trans*) which has no side chain; and (3) the corpus luteum and adrenal cortex hormones, unsaturated derivatives of pregnane (*cis*) and allo-pregnane (*trans*) which have ethyl side chains. Testosterone is produced in the testis, and estradiol and progesterone in the ovary, when these organs are stimulated by appropriate gonadotropic hormones of the anterior pituitary gland. Metabolic products of the sex hormones are found in the urine. All of the important sex hormones, except progesterone and adrenosterone, contain hydroxyl radicals; they can easily form *glycuronides* (ethers of glycuronic acid) and *fatty acid esters*. The glycuronides are excretory forms which are insoluble in fat solvents; the esters exert prolonged physiological activity, and are, therefore, used in therapy.

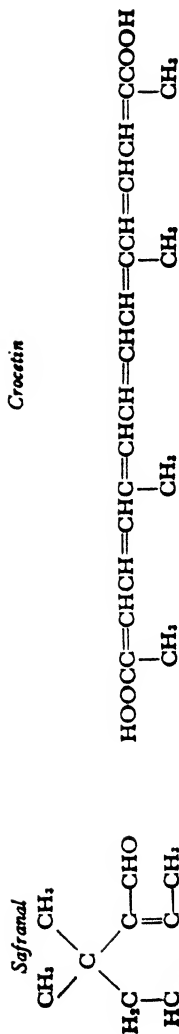
Estrogens, or Female Sex Hormones. Estradiol is a dihydroxy hormone of the ovary, found also in the placenta and in pregnancy urine. It is oxidized to a trihydroxy product, *estriol*, and a sterone, *estrone*, which are excreted in the urine. A similar dihydroequilenin series of more unsaturated hormones is found in mare pregnancy urine (Table 36, page 194). Equilin is formed from estrone in mares as pregnancy progresses. The estrogens are phenols, and can be oxidized by laccase.

These hormones are estrogenic, that is, they produce estrus changes in the female genital tract of mammals. The *international estrogenic unit* is equal to 0.1 γ of estrone. Estradiol and estradiol esters are more active than estrone. The proliferative changes of estrus show certain resemblances to those of malignant growth. Also, the ovarian hormones aid carcinogenesis in the mammary glands; and certain highly unsaturated sterols and carcinogenic hydrocarbons are slightly estrogenic. It is, therefore, possible that cancer is related to excessive or faulty unsaturation of sterides.

Estrogens are found in vertebrates, invertebrates, and arthropods. Estrone is present in the urine and testes of male animals. The latter may contain three hundred times the quantity present in the ovaries of the same species. Estrone and estriol have also been found in certain plants,

Vitamin A₂ (Tentative)

Crocefin



Hydroxy-β-Ionone has its —OH radical at •.
Hydroxy-α-Ionone has its —OH radical at ••.

¹ These carotenoids have structures similar to lycopene, except as indicated.

and estrogenic substances are present in bituminous material. Estriol is a more highly dissociated acid than is estrone; it can be separated from the latter by extraction of an ether solution with dilute alkali. Treatment of pregnancy urine with acid increases the yield of hormones from three to nine times, by hydrolyzing the ether-insoluble glycuronides.

While it is customary to assay sex hormones biologically, attempts have been made to use colorimetric methods. The phenolic radicals of estrogens can be coupled with diazonium salts (diazotized sulfanilic acid, *p*-nitroaniline), phthalic anhydride, or guaiacol sulfonic acid to form colored compounds. The sterones react with a modified Jaffe reagent (picric acid and *m*-dinitrobenzene) to give purple coordination compounds (Zimmermann reaction). A specific reaction for testosterone is the production of a green coloration in the presence of copper salt, concentrated sulfuric acid, and a solution of potassium guaiacolsulfonate.

Androgens, or Male Sex Hormones. The androgenic sterones are more saturated than the female sex hormones (Table 36, page 194). The hormone of the testis, *testosterone*, is converted into the less active excretory products *androsterone* and *dehydroandrosterone*. *Adrenosterone* is a steride of the adrenal cortex which has male sex hormone activity. The androgens control the development of secondary male genital organs and secondary sex characteristics. The *international androgenic unit* is equal to 100 γ of androsterone or 15 γ of testosterone.

Corpus Luteum and Adrenal Cortex Hormones. These hormones are derivatives of pregnane and allopregnane, which have ethyl side chains (Table 36). *Progesterone* is the corpus luteum hormone which prepares the uterus for implantation. The corpus luteum (or yellow body) is a carotene-containing tissue formed in the ovary after the ripening and rupture of the follicle. Progesterone is more specific in its action than the estrogenic and androgenic hormones; and a much larger dosage is required for physiological effects. It may be regarded as the only specific "female" sex hormone. The relations of progesterone to pregnancy are given on page 698. Progesterone is formed in the ovary and placenta. Its reduction products, pregnandiol and allopregnanediol, are excreted as glycuronides in the urine. Glycuronic acid unites with the hydroxyl radical at carbon 3 of pregnandiol. The *international unit* is equal to 1 mg. of progesterone (progestational activity in the rabbit).

The steride hormones of the adrenal cortex include *desoxycorticosterone*, *corticosterone*, *dehydrohydroxycorticosterone* (Compound E) and an amorphous fraction. Compound E can be oxidized to adrenosterone.

HYDROCARBONS AND FAT-SOLUBLE DERIVATIVES

Hydrocarbons

The biologically important hydrocarbons include those with straight or branched chains, and also the highly unsaturated and methylated fat-

soluble pigments known as *carotenoids*, *lipochromes*, or *polyene pigments*. The hydrocarbons are soluble in lipides and, since they are unsaponifiable, they accompany the sterols and fatty alcohols in lipide fractionation. Examples of aliphatic hydrocarbons are the liquid isoctadecane ($C_{18}H_{38}$) of liver oils, and the solid series typical of beeswax and plant waxes, namely, pentacosane ($C_{25}H_{52}$), hexacosane ($C_{26}H_{54}$), heptacosane ($C_{27}H_{56}$), octacosane ($C_{28}H_{58}$), nonacosane ($C_{29}H_{60}$), triacontane ($C_{30}H_{62}$), and hentriacontane ($C_{31}H_{64}$). Pentacosane and heptacosane have also been found in small quantities in pregnancy urine.

Liver oils contain appreciable quantities of unsaturated branched hydrocarbons, of which *squalene* is an interesting example. This hydrocarbon contains six methyl side chains and six unsaturated linkages, features which relate it to the biologically important hydrocarbons, the carotenoids (Table 37). Typical carotenoids are: *lycopene* (the red pigment of tomatoes, apricots, watermelons, and tropical fruits), and α -, β -, and γ -carotenes (yellow pigments of carrots, squashes, pumpkins, sweet potatoes, leaves, etc.). The structure of the carotenes is similar to that of the lycopenes, except that the former contain closed ionone rings (Table 37). Hydroxy derivatives of the carotenoid hydrocarbons include the vitamins A_1 and A_2 , and such plant pigments as *cryptoxanthin* and *zeaxanthin*, found in corn and egg yolk, and *xanthophyll*, or *lutein*, found in leaves and egg yolk. A great variety of carotenoids and their derivatives exist in roots, leaves, fruits, corn, banana skins, dandelions, and the yellow flowers of plants, and also in algae, crustacea, and colored bacteria.

The carotenoids have polyene, or isoprene, chains; that is, they are characterized by a system of conjugated double bonds ($-C=CH-CH=C-$). For this reason, the carotenoids are very reactive and, especially when impure, they are readily auto-oxidizable. They can be separated by chromatographic adsorption on columns of alumina, calcium carbonate, and so forth.

Carotenols

The hydroxy carotenoids, or *carotenols*, are easily esterified with fatty acids. They also unite with sugars to form glycosides. In animal tissues the carotenols are often united with proteins. The carotenes, echinenone, and cryptoxanthin, which contain the unsaturated β -ionone ring, are precursors of vitamin A in animals. The vitamin is produced by oxidizing the carotenoids at the midpoint of the carbon chain. Two β -ionone rings are present in β -carotene but only one occurs in the other provitamins, which, therefore, form only one half as much vitamin as does β -carotene. While carotenes are present in greatest concentrations in plant oils, the vitamin is found chiefly in animal fats and oils. Vitamin A_1 , *axerophthol*, predominates in the tissues of mammals, birds, and marine fishes, and in fish liver oils, while vitamin A_2 is more abundant in fresh-water fishes. Note that A_2 is a dehydrogenation product of A_1 (Table 37). The vitamin A of fish

liver oils exists partially as the palmityl ester. Mammalian liver oils contain an inactive divitamin A_1 , $C_{40}H_{58}(OH)_2$; this substance, known as *kitol*₁, and the similar *kitol*₂ can be activated by heating.

The vitamins A are colorless but they show characteristic absorption of ultraviolet light at 328 $m\mu$, by which they may be determined (spectrophotometrically). Another method of analysis is biological assay. In chloroform solution, vitamin A gives a temporary blue color with antimony trichloride (method of Carr and Price). This pigment has a specific absorption band which differentiates it from the similar color given by carotene. The method is, however, non-specific; many carotenoids show similar halochromism, both in the Carr and Price test and also in the presence of concentrated sulfuric acid. In the latter case, colored carbonium salts are formed. Vitamins A_1 and A_2 can be detected in tissues by illumination with ultraviolet light, which produces transient green and yellow-brown fluorescence, respectively. β -Carotene and especially vitamin A are destroyed rapidly in the presence of light and oxygen; their oxidation in fats is subject to the influences which affect rancidity. Many carotenoids are thermolabile. The *international unit* of vitamin A is equal to 0.6 γ of β -carotene (or 0.3 γ of vitamin A_1). The biological activities and metabolism of this vitamin are discussed on page 645.

The carotenoid radical of *rhodopsin* or visual purple, a conjugated protein of the outer segments of the retinal rods of marine fishes and higher vertebrates, is termed *retinene*₁. It is chemically related to vitamin A_1 , into which it can be converted by light and heat. The *porphyropsin* of the retinae of fresh-water fishes has a *retinene*₂ radical, related to vitamin A_2 . A derivative of vitamin A is also present in the conjugated protein, *iodopsin* or visual violet, of the cones. Rhodopsin increases the sensitivity of the eye to faint light; its absorption maximum is at 500 $m\mu$, as compared with 522 $m\mu$ for porphyropsin. When exposed to light, visual purple is transformed into "transient orange," and this pigment is subsequently changed to "indicator yellow" (visual yellow); the eventual products are yellow *retinene* and a protein. In the darkened eye, rhodopsin is regenerated. The structure and function of the fat-soluble chromophane pigments (green chlorophane, yellow xanthophane, and red rhodophane), found in the cones of birds and reptiles, are not known. They appear to be carotenoid esters; *astaxanthine* (reduced *astacin*) is the suggested precursor of the red pigment, and *lacertofulvin* of the yellow pigment.

Carotenoid Aldehydes and Acids

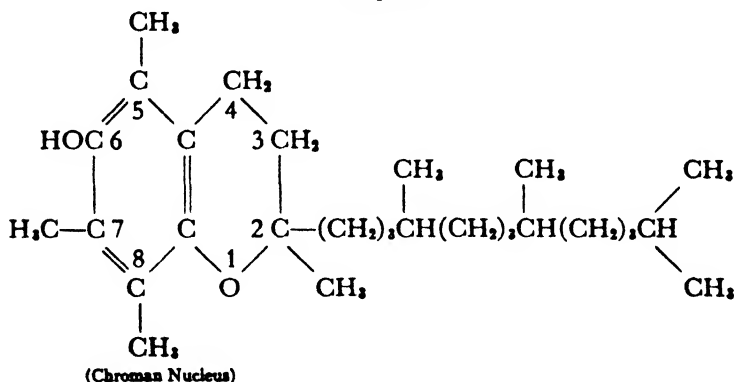
Safranal (Table 37, page 203) is a carotenoid aldehyde whose ring structure is similar to that of vitamin A_2 . It is a male-determining factor (or *termone*) for certain algae, while its glucoside, *picrocrocine*, is a female-determining factor. Green algae also contain a carotenoid dicarboxylic acid, *crocetin* (Table 37, page 203), which exists as *trans* and *cis* isomers. The correspond-

ing isomeric dimethyl esters are male and female conception hormones (or *gamones*). Crocetin forms similar esters with disaccharides (and in some instances, with trisaccharides); these esters, termed crocins, are exceedingly active stimulants of motility in algae. The crocins and picrocrocins are found in the reproductive apparatus of flowers.

Vitamins of the E Series

A fat-soluble vitamin, of importance in reproduction, is found in the nonsaponifiable fraction of plant oils, particularly in wheat germ oil. Three phenols have been separated and found to possess vitamin E activity. They are α -, γ -, and β -tocopherols, whose activities decrease in the order named; the *d* forms are the physiologically active stereoisomers. The functions of these vitamins are discussed on page 671. α -Tocopherol, or 5,7,8-trimethyltolcol, is a chroman derivative which is oxidized readily and has inhibitol properties (page 184). The natural isomer is dextro-rotatory. Its structure is as follows:

α -Tocopherol



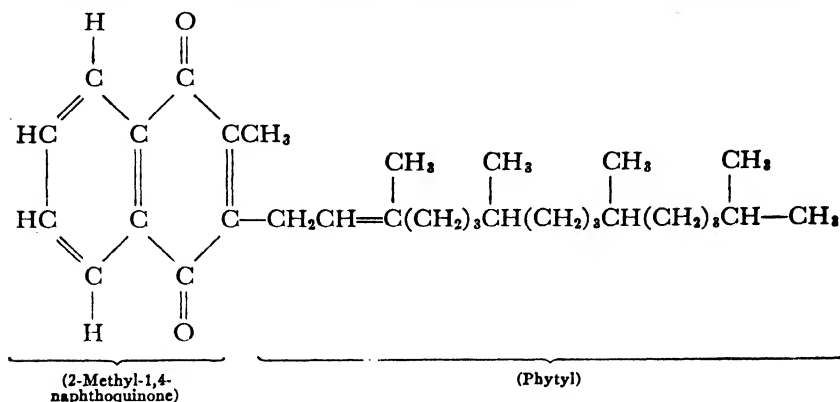
The *international unit* of vitamin E is equal to 1 mg. of *dl*- α -tocopherol acetate. The less active β -tocopherol (also termed cumotocopherol) is 5,8-dimethyltolcol, and γ -tocopherol is 7,8-dimethyltolcol. The side chain of the tocopherols is related to the phytyl side chain of vitamin K. (See following paragraph.) In the tocopherols, the phytyl side chain has united with a phenolic radical to form a heterocyclic ring. Tocopherols can be determined colorimetrically by means of ferric chloride and α,α -dipyridyl.

Vitamins of the K Series

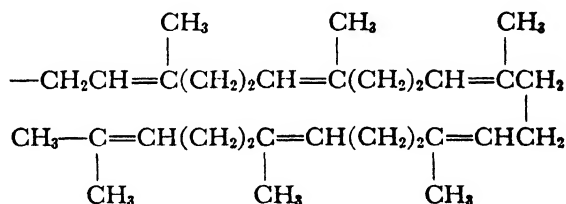
These fat-soluble vitamins are yellow colored derivatives of 1,4-naphthoquinone. The vitamins K are found in green plant tissues and in

vegetable oils; they are also synthesized by intestinal bacteria. Vitamin K₁ of plants has the following structure:

Vitamin K₁, 2-Methyl-3-Phytyl-1,4-Naphthoquinone, or α -Phylloquinone

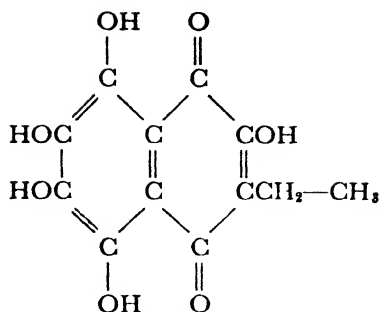


Vitamin K₂, a product of bacterial metabolism, is similar, but has a longer side chain,



at carbon 3. The phytyl radical, derived from phytyl alcohol, is also present in the green chlorophyll pigments of plant tissues. These vitamins are oxidized in the presence of alkali and are very labile to light. Phthiocol, a pigment formed by tubercle mycobacteria, is 2-methyl-3-hydroxy-1,4-naphthoquinone. The most active substances are the synthetic products, diphospho-2-methyl-1,4-naphthohydroquinone, 2-methyl-4-amino-1-naphthol, and 2-methyl-1,4-naphthoquinone. The latter substance, and related products, can be detected by the appearance of a green color, soluble in amyl alcohol, upon warming with 2,4-dinitrophenylhydrazine in hydrochloric acid and adding ammonium hydroxide. The *vitamin K unit* is equal to 1 γ of 2-methyl-1,4-naphthoquinone, or $3\frac{1}{3}$ γ of vitamin K₁. The relations of vitamin K to prothrombin metabolism have been discussed on page 65.

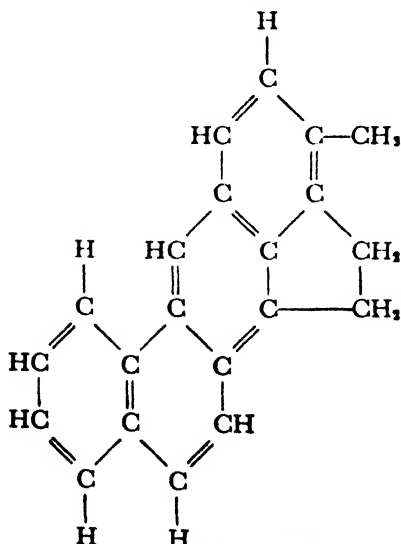
Reversibly reducible red substances, *echinochromes*, are found in the eggs of the sea urchin. Echinochrome A is related to vitamin K, as shown by its formula on next page. Echinochrome A is a gamone which attracts the spermatozoa of the sea urchin.



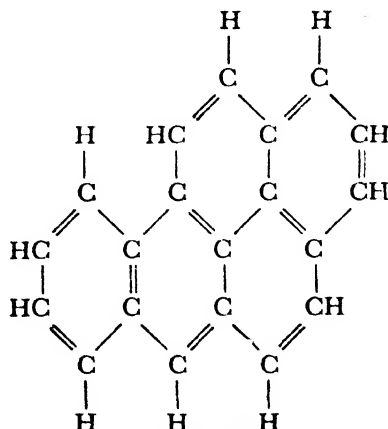
Echinochrome A

Carcinogens, Etc.

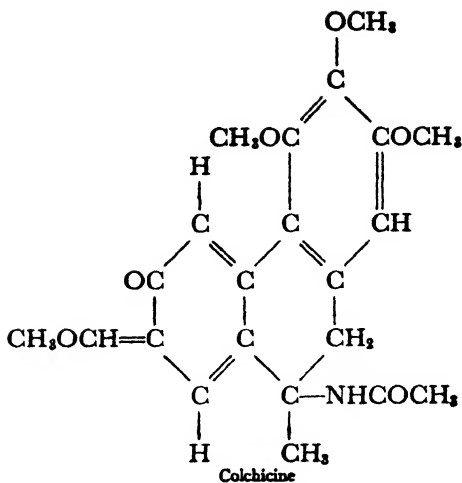
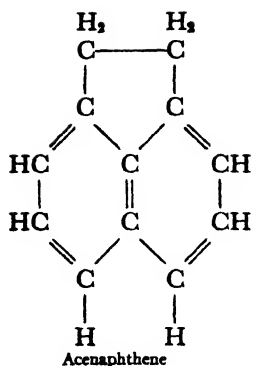
Mention should be made of a few synthetic carcinogenic or cancer-stimulating hydrocarbons. The most important of these substances are methylcholanthrene and 1,2-benzpyrene, whose structures are given below. *Methylcholanthrene* is the most active carcinogenic substance known. It can be synthesized, *in vitro*, from desoxycholic or cholic acid. Abnormal metabolism of bile acids, or their precursors, might conceivably be associated with the development of tumors. Subcutaneous injection of desoxycholic acid in sesame oil has been reported to produce spindle cell tumors in mice. 1,2-Benzpyrene is a potent carcinogen, present in tar. When applied locally, it incites the formation of skin epitheliomas; and when injected, it incites sarcomas of connective tissue. These hydrocarbons stimulate tumor formation, excessive mitosis, and precocious splitting in the prophase; they are also slightly estrogenic.



Methylcholanthrene



1,2-Benzpyrene



Certain aromatic azo compounds, such as *o*-aminoazotoluene and dimethylaminoazobenzene (butter yellow), stimulate carcinogenesis in the liver, while estrin can aid the production of mammary carcinomas.

Acenaphthene and a phenanthrene derivative of plant origin, *colchicine*, are caryotoxic substances which affect the spindle apparatus and arrest mitosis in the metaphase. They cause multinucleation and polyploidy (page 498). Sulfanilamide has a similar effect on plants. *Traumatic acid* is a dibasic acid whose formula is: $\text{HOOCCH}=\text{CH}(\text{CH}_2)_8\text{COOH}$. It is a "wound hormone," liberated by injured plant tissue, which induces division of normal mature cells.

METABOLISM

"Metabolism is never visible, except to the eye illuminated by all sorts of ideas." — MORRIS R. COHEN

This section includes the metabolism of fats, phospholipides, glycolipides, sterols, and bile acids. The functions of the fat-soluble vitamins and hormones are given in Chapters IX and X, respectively.

FATS

Food Fats

The human diet contains approximately 80 gm. of fat per day. The common foods with highest fat content (Table 38) are salad oils, bacon, nuts, chocolate, certain meats, egg yolk, and dairy products (butter, cream, and cheese). The food lipides consist largely of neutral fats, whose compositions have been given in Table 32, page 185. In America, most

TABLE 38

APPROXIMATE FAT CONTENT OF COMMON FOODS¹

	PER CENT FAT
Frying fats, salad oils	100
Butter, oleomargarine, salad dressings	85
Bacon, pecans, walnuts	65
Chocolate, peanuts, peanut butter	50
Heavy cream, potato chips	40
Cheese, coconut, egg yolk, goose	35
Ham, medium cream, pork	30
Avocado, light cream, ripe olives, sardines	20
Lean meat, salmon, sweetbreads	15
Baked beans, cakes, crackers, eggs, ice cream, green olives, pie, zwieback	10
Cocoa beverage, cream soups, dried beef	7
Fish, puddings	5
Liver, milk, spinach	4
Raisins, tea and coffee beverages	3
Bread, breakfast cereals, macaroni	2
Buttermilk, common vegetables, cottage cheese, egg white, fruits, jellies, noodles, oatmeal, rice, shellfish, soups, sugar candies	0-1

¹ These values apply to cooked, but not to fried, foods.

dietary lipide comes from animal sources, about one half from meat, and one quarter from dairy products. Vegetables provide one eighth, the cereals and fruits even less. Eighty per cent of the meat lipides come from pork. Approximately 25 per cent of the food fats are lost during the preparation of food.

Gastric Digestion

Dietary lipides are first subjected to digestion in the stomach, where the gastric lipase exerts a slight hydrolytic action on the highly emulsified fats of milk and egg yolk. Gastric digestion of fats is most rapid in infants, inasmuch as the optimum pH of gastric lipase coincides with the pH of infant gastric contents. However, the action of gastric lipase is so feeble that, even in the infant stomach, only 5 per cent of food fats are hydrolyzed. In adults, regurgitation of duodenal contents, which raises the pH of the gastric contents and introduces intestinal lipases, allows a small amount of fats to be digested. As mentioned previously, dietary fat delays gastric evacuation, and the fats with high melting points are most effective. Gastric digestion of fat is slightly increased by prolonged retention of food in the stomach. While digestion of lipides in the gastric cavity is of little significance *per se*, it does provide small quantities of fatty acids which assist in the subsequent emulsification of fats in the intestine. Gastric digestion of the protein membranes surrounding food fats indirectly aids subsequent intestinal digestion of the fats.

Intestinal Digestion

Food fats are very completely digested in the normal human intestine. The extent of digestion and absorption tends to vary inversely with the melting points of the dietary fats and directly with their unsaturation. Efficient digestion of food fat by steapsin and intestinal lipase requires emulsification, that is, conversion to tiny lipide globules which present large surface areas for adsorption of the water-soluble lipases. Emulsification is accomplished chiefly by the bile salts, although phospholipides, proteins, traces of soap (formed from the interaction of fatty acids and cations), and the movements of the intestine assist this process. Emulsification of fat is difficult at the acidity of gastric contents, but it is readily accomplished after the pH of the chyme is increased to 6 or more by being mixed with the intestinal juices.

Steapsin speedily hydrolyzes emulsified fats to glycerol and fatty acids. The necessity of this pancreatic lipase is demonstrated by the appearance of from one half to three fourths of the food fat in the feces following exclusion of the pancreatic juice. Under these conditions, the more highly emulsified food fats continue to be partially digested by gastric and intestinal lipases. Fat digestion is also intimately dependent on the presence of bile; the bile salts are important not only for the emulsification of fats prior to digestion, but also for the solution of insoluble fatty acid digestion products. These biliary functions are peculiar to lipide digestion; they do not apply to the digestion of carbohydrates or proteins. Inadequacy of either bile or pancreatic juice, therefore, interferes with normal fat digestion.

Food fats which escape digestion or absorption in the upper intestine are largely hydrolyzed by lipases of the succus entericus and by colonic bacteria. However, digestion in the lower intestine is too late for effective absorption of the fatty acids. It has already been stated that normal fecal lipides consist of unabsorbed fractions of intestinal secretory products. These endogenous fecal lipides are secreted largely by the small intestine, and also by the colon and the liver.

Bile salts dissolve fatty acids by forming coordination compounds. They dissolve unsaturated fatty acids, or mixtures, much more readily than pure saturated acids. Ninety per cent or more of administered tristearin is lost in the feces. Unsaturated fats are found in large amounts in plant and animal oils which are, therefore, important components of the diet. Their ingestion assists in the absorption of fat-soluble vitamins, carotenoids, sterols, and so forth. Since fats are the most difficult to digest of normal foods, they are frequent dietary causes of indigestion.

Absorption and Resynthesis

On diets containing from 100 to 400 gm. of fat, the daily fecal losses are usually in the neighborhood of 3 per cent, but at times they may be as

much as 8 per cent. After prolonged feeding of excessively high fat diets, the fecal loss may increase to 35 per cent of the intake. Certain hydroxy fatty acids, particularly ricinoleic acid from castor oil, are incompletely absorbed because of their cathartic effect.

Fat absorption is limited to the small intestine; its rate is related to the speed of gastric evacuation, the pylorus tending to remain closed as long as undigested fat is present in the duodenum. Absorption of fats is notably slower than that of other foods. As the glycerol and the water-soluble coordination compounds of bile salts and fatty acids are formed in the upper intestine, they diffuse into the mucosa and enter into a series of synthetic reactions in the epithelial cells. Resynthesis of fats in the mucosa is clearly indicated by the fact that ingested fatty acids, soaps, or mono-glycerides appear in the intestinal and thoracic lymph as neutral fats. The latter have been shown, by analysis and by staining, to accumulate in the intestinal mucosa during absorption. Within one-half hour after a meal, fatty acids are found in the epithelial cells at the tips of the villi. They can also be demonstrated in the enteric lymph; but near the external or striated border of the intestinal epithelial cells they exist as unstainable choleic acid complexes. During the second postprandial hour, the intestinal synthesis of neutral fat is well advanced; the intracellular droplets enlarge, and a high gradient is established for the diffusion of choleic acid complexes. By the sixth hour, intracellular fatty acids have practically disappeared from the mucosa, the cells are filled with neutral fat and the mucosa, serosa, and lymphatics are milky from their load of resynthesized fat.

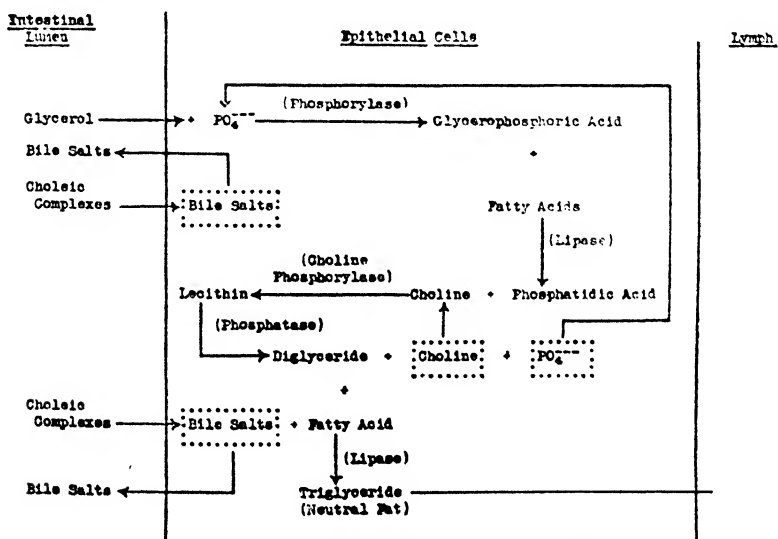


FIG. 3. Intestinal resynthesis of neutral fat.

The chemical processes which occur in the intestinal mucosa during the resynthesis of fat are outlined in Figure 3. After being dissociated from their bile salt coordination compounds, the fatty acids react with glycerophosphoric acid to form phosphatidic acids. The glycerol required in this reaction is partly absorbed and partly synthesized from glucose by the epithelial cells. The nitrogenous alcohols, choline and ethanolamine, then combine with the phosphatidic acids to synthesize the phospholipides. This synthesis is effected by intracellular lipase and phosphorylase. The intermediate phospholipides are, in turn, converted into neutral fats. While all of the details are not proved, there is much suggestive evidence for this mechanism of resynthesis. When foreign fatty acids, such as elaidic acid or iodized fatty acids, are fed, they can be found in the intestinal wall partially transformed to phospholipides. Mixtures of fatty acids and glycerophosphates are absorbed more rapidly than are fatty acids alone. Parenteral injection of moniodoacetic acid, or of large doses of phlorhizin, inhibits the phosphorylation mechanism and hinders fat absorption seriously. The diffusion of the fatty acid complexes reaches an equilibrium because of inhibition of resynthesis. Riboflavin deficiency, adrenalectomy, anesthesia and oxygen deficit interfere with fat resynthesis. An actively metabolizing mucosa is, therefore, necessary for normal fat absorption.

Since an influx of large quantities of fatty acids is somewhat toxic to animals, resynthesis may be considered as a detoxicating mechanism. During the synthetic process, the fatty acid units of foods are saturated or unsaturated to some extent and the composition of the resynthesized fat is modified toward that of the depot or storage fat of the species. Owing to the relatively low pH of intestinal contents, only traces of soaps are formed in the intestinal lumen. Soaps given *per os* are neither absorbed nor transported as such; intravenous injection of 15 mg. of sodium oleate per kg. of body weight is toxic to rabbits. Symptoms of the intoxication include pronounced fall in blood pressure, incoagulability of the blood, and coma.

Lymphatic Transport

Resynthesized fats pass readily into the central lacteals of the intestinal villi, and the major portion (over 60 per cent) is transported in the fatty lymph or *chyle* through the thoracic and accessory ducts to the left jugular vein. After fatty meals, the chyle at the thoracic duct contains 2 to 8 per cent of lipide. The chylous lipides are largely neutral fats, but some phospholipides, cholesterol, cholesterol esters, and fatty acids are also present. Administration of triolein causes a marked increase in the lipide content of the enteric lymph; the neutral fat is accompanied by approximately one tenth as much phospholipide, formed in the intestinal epithelial cells. There is not a comparable lymphatic transport of protein and carbohydrate digestion products; these pass almost entirely through the portal circulation. The lymphatic diversion of fat prevents a sudden portal

influx of neutral fat, which causes derangement of liver metabolism. When either the thoracic duct or the enteric lymph vessels are ligated, fat absorption is impeded, as demonstrated by the absence of systemic lipemia, but it is not altogether abolished. Under these circumstances, some resynthesized fat is transported to the systemic circulation via the portal blood. The livers of such animals show fatty degeneration. When emulsified fats are injected parenterally, they pass slowly into adjacent lymphatics prior to entering the blood; intravenously injected fat is removed rapidly from the blood. In starving animals, neutral fats are transported via the lymphatics from the storage depots to the general circulation. It is evident that the lymphatic circulation has an important general function in the gradual delivery of lipides to the systemic blood.

Blood Fat

Normal values for human blood lipides, after a night's fast, are given in Table 39. In addition to the tabulated fractions, there are traces of free fatty acids in blood plasma. Plasma lipide values serve as clinical standards, inasmuch as most of the hematic lipide transport occurs in the plasma. In many species, including man, a variable portion of blood lipides exists as microscopically visible droplets — the *chylomicrons* or *hemoconia*. The remainder is present in invisible colloidal dispersion.

TABLE 39
AVERAGE NORMAL BLOOD LIPIDES¹
(Mg. Per Cent)

	CHILD	ADULT	
	Plasma	Plasma	Erythrocytes
Total lipide ²	520	720 ± 200	720
Neutral fat ³	100	225 ± 140	170
Phospholipides (as lecithin)	135	200 ± 40	350
Total cholesterol	190	200 ± 50	200
Free cholesterol	45	55 ± 15	200
Ester cholesterol	145	145 ± 35	Trace
Cerebroside		Trace	35

¹ Average iodine number of blood lipides = 87.

² The blood lipide values of the newborn are much lower.

³ Total lipide is approximately equal to the sum of neutral fat, phospholipide, total cholesterol, and the fatty acid equivalent to the esterified cholesterol.

⁴ Ordinary values, which include cerebroside.

An increase of plasma lipides above normal levels is termed *lipemia*. It represents the entrance of lipide into the blood faster than it can be re-

moved. The serum often assumes a milky appearance due to the increased number of chylomicrons. This condition is known as *lactescence*. After meals of neutral fat, the blood lipides gradually increase to a maximum at the fifth or sixth hour and then return to normal values in about nine hours. Exercise decreases this alimentary lipemia, and intravenous heparin abolishes it in dogs. Phospholipides and sterol esters of the plasma rise during alimentary lipemia, the former representing about 15 per cent of the total lipide increase. The formation of phospholipides and sterol esters may assist in the transport of neutral fats. When elaidic acid is fed to rats, it appears as a unit of the plasma phospholipides during the subsequent alimentary lipemia. Absorbed fat, therefore, circulates in blood plasma largely as neutral fat and phospholipide.

While a portion of the postprandial blood lipides originates from resynthesis in the intestine, the liver and other body tissues also contribute plasma lipides, from time to time, and thus aid in the control of blood lipide levels. Starvation, lactation, and abnormalities of carbohydrate utilization lead to lipemias which are not of alimentary origin. The injection of insulin and thyroidectomy lower blood lipides, while administered adrenaline causes a small rise in neutral fat. Injection of estrogen can induce intense lipemia in fowls, but it has a much smaller effect in man. An undetermined factor in the pancreas and pancreatic juice can assist the maintenance of the blood lipide level.

The blood phospholipides and the serum proteins permeate capillary endothelia more readily than they do the intestinal mucosa. The general tissues assimilate lipides as needed, while the liver, adipose tissues, and the mammary glands remove them rapidly for specific purposes. Blood lipides of the chick embryo gradually increase until the last seventh of embryonic life, then fall until after hatching. Plasma lipides are low in the human fetus at term, and in the newborn infant. Neutral fats stained with Sudan III, and "labeled" fats do not penetrate the placenta readily.

Lipides are found in certain pathological effusions. Escape of chyle produces *chylous effusions*, which contain considerable protein in addition to lipides and tend to clot spontaneously. *Chyliform effusions* originate through fatty degeneration of adjacent tissues.

Storage in Adipose Tissue

Active tissues metabolize the fat which comes to them, while adipose tissues normally store neutral fat and balance the dietary supply against the demands of the metabolizing tissues. The chief storage depots are in the subcutaneous connective tissue, intermuscular connective tissue, the omentum, mesentery, and adipose tissues in the region of the heart, kidney, liver, lung, ovary, and testes. Fat is also stored in the interstitial tissues of all organs, except the brain. About one half of the normal adult storage is in subcutaneous connective tissues. The anatomical distribution

of stored fat is affected by endocrine and nervous influences. Fat first appears in adipose cells as tiny globules, which later coalesce and fill the cells.

The melting points of stored fats vary with the species, but the fat of any one species is relatively constant in composition. Human adipose tissue fat consists chiefly of triglycerides of linoleic, oleic, palmitic, and stearic acids (Table 32, page 185). It is slightly yellow from dissolved carotenoid pigments (carotene, lutein, lycopene, vitamin A, etc.). Depot fat contains only very small amounts of the more unsaturated auto-oxidizable fats. During the last third of embryonic life, fat is deposited in the connective tissue and in the liver. This fat is more highly unsaturated than adult depot fat. The relatively low fraction of oleyl glyceride in the storage fat of infants increases to the characteristic adult value during the first year of life.

A normal infant has approximately 650 gm. of adipose tissue, the adult man 10 kg., and the adult woman 12 kg., representing 20, 15, and 21 per cent of the respective body weights. Hypothyroidism, hypogonadism, and certain types of hypopituitarism and nervous pathology increase fat deposition. The injection of insulin increases appetite and fat storage. Thyroxine administration decreases fat storage by stimulating energy production, and it causes greater unsaturation of the remaining stored fat. Hereditary influences and individual habits also affect fat deposition. Apart from these factors, fat storage is largely determined by the balance between caloric intake and energy expenditure. Excessive caloric value of the diet encourages storage; exercise decreases all but the intermuscular deposits. During prolonged starvation, 97 per cent of the fat of the storage depots can be withdrawn. After the second day of starvation, the adipose tissue provides from 75 to 90 per cent of the body's energy and ketosis appears (page 37). The 9 kg. of fat stored in the adult man can provide about 1300 calories per kg. of body weight, or sufficient energy for more than seven weeks of life.

Neutral fat is, thus, the principal reserve foodstuff of the body; the storage of carbohydrate and protein is much more limited. Neutral fat is less readily oxidized than other lipides, proteins, or carbohydrates; and it tends to accumulate on excessive diets. Fat storage is favored by diets high in fats or carbohydrates. The deposition of fat is accompanied by relatively little water storage, whereas deposited protein and carbohydrate retain about three times their weight of water. Hence, fat provides 8 calories per gram of tissue storage as compared with 1 or 2 calories for either carbohydrate or protein.

The composition of depot fat varies somewhat with abnormal diets, owing partly to limitations in the ability of the intestinal mucosa to saturate or desaturate fats during resynthesis. However, the rate of fat storage is not appreciably affected by changes in the nature of dietary fats. Deutero fatty acids have been used to trace the metabolic paths of fatty acid units.

Isotopes which have a sufficiently long half life may be used to prepare labeled compounds for metabolic experimentation. Since small concentrations of isotopes are metabolized by living cells in the same manner as their ordinary analogues, they can be used to trace metabolic paths. The substance used must contain less than 10 atoms per cent of the isotope, substituted at a stable linkage, as, for example, deuterium (heavy hydrogen) in $-\text{CH}_3$ and $=\text{CH}_2$ radicals, S or N isotopes bound to carbon, and S and P isotopes in sulfates and phosphates. Deuterium in polar radicals such as $-\text{OH}$, $-\text{COOH}$, and $-\text{NH}_2$, or attached to carbon atoms adjacent to a ketone radical, is interchangeable with the hydrogen of water. Oxygen isotope in the carboxyl radical is labile.

The deuterium content of an analytical sample is determined by oxidizing the organic material to water, and measuring the density or the refractive index of the latter. In the case of the isotope N^{15} the sample is oxidized, by the Kjeldahl procedure, to ammonium sulfate, the ammonia is converted to nitrogen by alkaline hypobromite, and the N^{15} content is determined by the mass spectrometer. Such radioactive isotopes as C^{14} , Fe^{59} , P^{32} , and S^{35} may be determined by the Geiger counter. These methods detect marker or tracer isotopes in dilutions up to 0.1 atom per cent.

Aside from their value in metabolic experiments, the isotopic compounds can be used for the quantitative determination of substances in biological mixtures. For this purpose, a definite quantity of the isotopic analogue of the desired compound is added to a quantitative aliquot of the unknown. Then, a portion of the desired substance is separated by any convenient chemical procedure and its isotopic content is determined. A simple calculation predicts the original quantity of the compound in the unknown. This method will undoubtedly prove very valuable for the determination of fatty acids, amino acids, and so forth.

Experiments with deuterio fatty acids have demonstrated that higher fatty acids are rapidly and extensively deposited, both in the fat depots and in internal organs. At least one half of the absorbed fatty acid units are stored in the adipose tissues, even in animals on restricted caloric intake and with no gain in weight. Fatty acid units are, therefore, retained by adipose tissue prior to their oxidation. The dietary fatty acids are continually replacing the fatty acid units of body fats. The lipases of the cells constantly accelerate lipide synthesis and hydrolysis. In adult mice, one half of the total body fat is regenerated within a week; the half life of the depot fatty acid units is 6 days, as compared with 3 days for those in the liver. One half of the saturated fatty acid units of the liver lipides can be regenerated daily; only the highly unsaturated fatty acid units are static.

When starved animals are fed fats which contain abnormal fatty acid units (erucic, myristic, elaidic, or iodized acids), these are incorporated into the adipose tissue fat. Experiments with deuterio butyric and caproic acids have shown that fatty acid units which have less than ten carbon atoms (acids characteristic of butter and coconut oil) are not deposited but are destroyed quickly. Injected sodium acetate, containing C^{14} in the carboxyl radical, readily liberates carbon dioxide which contains the heavy carbon. Hogs fed on low fat rations, such as corn or skimmed milk,

deposit lards with relatively high melting points and low iodine numbers. Herbivorous animals, whose diets are high in carbohydrate, store tallows of high melting points.

Since the adipose fat is maintained in a fluid state, *in vivo*, its composition is necessarily related to body and environmental temperatures. In the same animal, the melting points and composition of individual deposits vary with the location; internal deposits have higher melting points and lower iodine numbers than those of the subcutaneous layers. In infants, the higher melting point of the depot fat corresponds to the higher body temperature.

The general tissues remove lipides from the blood stream, but the chief lipides of these cells are phospholipides and sterols. The lipides of non-adipose tissues are regarded as metabolically active protoplasmic constituents, and have been named the *élément constant*. (See phospholipide metabolism, page 231.) The lipides of the general tissues have little storage significance; they are much more constant in quantity than the fluctuating adipose stores, or *élément variable*. However, the lipides of the general tissues are continually regenerated as shown by exchange of their fatty acid units with administered isotopic higher fatty acids. The liver is the most active tissue in this respect. Muscle lipides partially resemble adipose stores; they increase somewhat after excessive diets and decrease during starvation.

Role of the Liver; Lipotropic Factors

The liver has long been considered important in the intermediate metabolism and mobilization of lipides. In addition to forming the bile acids, the liver unsaturates fatty acid units of lipides, phosphorylates neutral fats to produce phospholipides, and effects exchange esterification between fats and sterols. Normal human liver contains from 3 to 8 per cent of total lipides; in terms of fatty acid units, there are three parts of phospholipide to two of neutral fat. The fatty acids from beef liver fat have an average iodine number of 87; those from the complex lipides have an iodine number of 110. The lipide content of the liver is markedly increased during the fatty infiltration and degeneration produced by starvation and by certain pathological processes. During fatty infiltration, neutral fat exceeds the phospholipides in the liver and its influx may raise the liver lipide content to as much as 20 per cent. The excess neutral fat has a markedly low iodine number.

Feeding cholesterol to rats results in hepatic accumulation of both neutral fat and cholesterol esters. Diets high in fat and low in protein cause an increase in liver lipides having comparatively low iodine numbers. Depancreatized dogs, maintained with insulin on lecithin-deficient diets, develop jaundice and fatty livers. Raw pancreas, or alcoholic extracts of the organ, relieve the pathology. This effect has been attributed to a special pancreatic hormone (lipocaic), which is produced by the α

cells of the islands of Langerhans. Lipocaic can counteract the fatty infiltration produced by fasting. It is now established that dietary choline, phosphocholine, lecithin, betaine, methionine, and protein, known collectively as *lipotropic factors*, prevent certain types of fat deposition in the mammalian liver and promote phospholipide synthesis in this organ. Dietary deficiency of the lipotropic factors causes the liver to synthesize extra neutral fat from carbohydrate, as shown by experiments with deuterio fatty acids. This hepatic synthesis of fat is accompanied by some infiltration. Choline or inositol administration reduces the fat content and exerts a small lipotropic action on the cholesterol esters of fatty livers; choline and methionine do not completely relieve the hepatic fatty degeneration which follows cholesterol feeding. Neither choline nor lipocaic prevents the fatty infiltration of the liver produced by phosphorus or carbon tetrachloride poisoning or by administration of excess anterior pituitary extract, estrone or pitressin, conditions in which there is a shift of fat from the depots to the liver (as proved by studies with deuterio fatty acids). Fatty infiltration is also the principal factor in the production of fatty livers in clinical patients. Betaine and the phosphorus and arsenic analogues of choline have somewhat more than one half the lipotropic activity of choline. Ethanolamine and the choline units of tissue phospholipides are not available for lipotropic action.

Fatty infiltration of the liver is related to the amount of protein in the diet. One gm. of casein is equal to only 6.5 mg. of choline, and a 30 per cent dietary level of protein is necessary for optimal lipotropic action. Ovalbumin, beef protein, edestin, fibrin, gliadin, gelatin, and zein have smaller lipotropic effects, decreasing in the order named. The amino acid, methionine, has about one twelfth to one fifth the activity of choline and it is at least partly responsible for the lipotropic action of dietary protein. The lipotropic action of methionine is traceable to its transmethylation activity in the synthesis of choline. Cystine has an opposite effect; and 2 per cent nicotinamide in the diet causes fatty livers by the excessive use of methyl radicals for detoxication. Levels of dietary protein which effectively diminish infiltration of neutral fats tend to increase the cholesterol ester content of the liver.

The several conditions which incite fatty infiltration and degeneration of the liver disturb either the transport equilibria of lipides or the synthesis of neutral fat from carbohydrate. Thiamin is necessary for fat synthesis from carbohydrate, and both thiamin and pyridoxin are concerned in the synthesis of fat from protein. The administration of thiamin increases the fat content of the livers of choline-deficient animals, and, vice versa, an increased fat and choline intake reduces the thiamin requirement. Inositol has no effect on fatty livers produced by thiamin. Biotin (vitamin H) can stimulate fat synthesis and the production of fatty livers in rats; this activity is counteracted by lipocaic or inositol but not by choline. It is claimed that **adrenal cortical hormones are necessary for certain fatty infiltrations of the**

liver. Maintenance of normal lipid metabolism of the liver requires not only dietary lecithin (choline), cholesterol, neutral fat, and protein, but also a liberal supply of carbohydrate and its normal utilization. As discussed more fully in the section on lipid pathology, fatty degeneration of the liver is frequently associated with decreased hepatic glycogen. In fact, the adequate administration of carbohydrate and protein is, at present, the most successful therapeutic measure for the management of clinical liver degenerations. The fatty livers of diabetic patients, for example, are due not to deficiency of lipotropic substances but to abnormalities of carbohydrate metabolism. It has been reported that fatty infiltration of the liver in phlorhizinized animals is prevented by section of the cervical cord.

Unsaturation and Phosphorylation

Many tissues unsaturate and phosphorylate neutral fats, but the liver is especially active in this respect. Deutero palmitic and deutero stearic acids given *per os* are found partly as deutero palmitoleic and deutero oleic acid units in the tissue lipides. This unsaturation of fatty acid units of lipides is a type of dehydrogenation that is occurring continually in the tissues, together with the reverse process of saturation. After fatty meals, and even after the ingestion of highly unsaturated liver oils, the tissue lipides become more unsaturated than the administered fat. While certain unsaturated fatty acids (oleic and palmitoleic acids) are formed readily, linoleic and linolenic acids are dietary essentials of mammals. Experiments with deutero derivatives show that these fatty acids are not easily formed by the unsaturation mechanism of the tissues. Rats whose diets contain neither linoleic, linolenic, nor more highly unsaturated fatty acids lose weight and develop skin and kidney lesions. Linoleic acid is especially effective in preventing this fat-deficiency disease; also active, in decreasing order, are arachidonic, linolenic, and the more unsaturated fatty acids of cod liver oil. The low iodine number of the plasma lipides of young dogs on a minimal fat intake, and of certain human eczematous subjects, is attributed to decrease in the linoleic and arachidonic acid fractions. The highly unsaturated fatty acid units of liver lipides, such as arachidonic and clupanodonic acids, are evidently formed from the linoleic and linolenic acids of the diet. Pyridoxin (vitamin B₆) assists in the utilization of the essential fatty acids (page 659).

Phosphorylation is another general function of tissues, which is coupled with oxidation reactions. It is therefore inhibited by cyanide, hydrogen sulfide, or anaerobiosis. The phospholipides can be synthesized independently of the liver, as, for example, in the intestinal epithelium during fat absorption. It was at one time believed that hepatic unsaturation and phosphorylation of fats were essential to their subsequent oxidation in the general tissues; but it is becoming increasingly evident that these liver processes are specialized regulatory phenomena, and not obligatory pre-

requisites for fat oxidation. Hepatectomy has little effect on fat oxidation. Also, ingested saturated fat does not produce a sufficient increase in the iodine number of tissue phospholipide to indicate obligatory desaturation and phosphorylation. The unsaturation of liver lipides (and, to a smaller extent, of muscle phospholipides) which follows a very small intake of cod liver oil is far in excess of that which might be required for the oxidation of the small quantity of food oil.

While unsaturation and phosphorylation are functions of dehydrogenases and phosphorylases in the general tissues, there is an especially marked lipid turnover in the liver. It is significant that the highly unsaturated liver lipides contain chiefly C_{20} and C_{22} fatty acid units, whereas the main dietary units are C_{16} and C_{18} fatty acids. The liver normally exerts an important influence upon desaturation, phosphorylation, and exchange esterification of fats in the body. However, these processes are more closely related to the transportation and mobilization of fats than to oxidation in the general tissues.

Oxidation

Fat constitutes only 15 per cent of the dry weight of the average human diet; but, owing to its high caloric value of 9.3 calories per gram, its oxidation normally provides approximately 25 per cent of the daily energy. During starvation, or carbohydrate deprivation, the oxidation of fat is responsible for from 80 to 90 per cent of the body energy. Thyroxine stimulates the oxidation of fats as well as of other foods. Fat is only slightly inferior to carbohydrate as a source of energy for moderate muscular work; but, in strenuous activity, carbohydrate is essential to delay exhaustion. Severe exercise lowers the blood sugar level; the blood sugar is rapidly restored by the administration of carbohydrate, but not by fat. Exercise produces a slight lipemia in fasting men, because of mobilization of fat from the storage depots and its transport in the blood plasma to the active muscles. This lipemia is abolished by feeding glucose prior to exercise.

During absorption and transportation, fat exerts a slight specific dynamic action, occasionally as much as + 25 per cent at the end of the sixth postprandial hour, but only + 4 per cent for the total period. Terrestrial embryos oxidize relatively large amounts of fat during late embryonic life. Fat is a convenient form of energy storage in terrestrial eggs; its oxidation leaves only carbon dioxide and a considerable quantity of the water so important to avian embryos.

Administered deuterio cetyl and deuterio octadecyl alcohols are absorbed, and are oxidized rapidly by mammals to deuterio stearic and deuterio palmitic acids. The latter can also be reduced to the corresponding deuterio alcohol, small portions of which are excreted in the feces.

The β -oxidation of fatty acid units of lipides, in living cells, was demon-

constitutes, at present, the principal reason for supposing that fatty acids are oxidized in the form of phospholipides. The phospholipides of the brain and testis apparently are not involved in fatty acid oxidation.

In the intact animal, synthetic fatty acids having uneven numbers of carbon atoms are degraded by β -oxidation; the resulting C_3 fragments can be converted to glucose and deposited as glycogen in the liver. α -Methylated and dicarboxylic fatty acids also undergo β -oxidation; succinic acid (C_4) is the only dicarboxylic fatty acid which is appreciably oxidized in humans. Unsaturated fatty acids are not oxidized at the unsaturated linkages, unless these represent loci for β -oxidation. There have been suggestions that bile salts function through coordination linkages at alternate carbon atoms of fatty acids to orient β -oxidation; but the presence of bile salts in the general tissues is not proved.

In the body, most dietary fat is oxidized by β -oxidation; a small fraction (less than 1 per cent of the total) may undergo ω -oxidation at the $-\text{CH}_3$ ends of the fatty acid chains. In man, fatty acid units with from 8 to 12 carbon atoms can be partially ω -oxidized to dicarboxylic acids which are excreted in the urine.

Ketogenesis

Acetoacetic and β -hydroxybutyric acids are produced in small quantities by normal animals. Since their tissues can oxidize such limited quantities, only traces of acetone bodies are present. Normal human blood contains about 2 mg. per cent of total acetone bodies. Man can utilize approximately 35 mg. of acetoacetic acid per kg. of body weight per hour. Brain and liver do not participate in this process, and the mammary gland can utilize β -hydroxybutyric but not acetoacetic acid. The rate of acetoacetic acid utilization in muscle increases with the acetone body level of the blood. Exercise apparently increases both oxidation and synthesis of acetone bodies in ketosis patients. The eviscerated depancreatized dog can dispose of injected acetoacetate at the same rate as the normal extrahepatic tissues. The oxidation paths of the acetone acids, and the final stages of normal fatty acid catabolism, are interlinked with those of carbohydrate intermediates.

Accumulation of acetone bodies is a sign of disturbed carbohydrate metabolism. It was formerly believed that fatty acids were degraded by β -oxidation to the acetone body stage, at which point normal utilization of carbohydrate was necessary for oxidation of the butyric acid derivatives to carbon dioxide and water. Revision of this concept was necessary following the demonstration that hepatectomy lowers the acetone body level of the blood in diabetic dogs, and that the liver is responsible for ketosis and ketogenesis.

Synthesis of acetone bodies from acetic acid (probably by way of acetyl phosphate) has been shown in fasting rats by use of the isotope, C^{14} . Liver slices from these animals produce acetoacetic acid containing C^{14} at both

the carboxyl and β positions when incubated with a fatty acid having the isotope in the carboxyl radical only. Acetone bacteria use both acetic and pyruvic acids as precursors of acetone. Similarly, mammalian liver can synthesize acetoacetate from pyruvate, a reaction which is accelerated by thiamin. Such investigations indicate that hepatic ketogenesis is a synthetic process which involves condensation of two-carbon intermediates from fatty acid oxidation, or of carbohydrate intermediates.

Observations on diabetic animals and surviving liver preparations show that acetoacetic acid synthesis is stimulated much more by natural fatty acids than by those having an odd number of carbon atoms. The natural fatty acids with less than 12 carbon atoms are oxidized readily in animals, and they induce greater ketogenesis than equal weights of the higher fatty acids. Commonly employed dietary calculations assume the formation of one mol of acetoacetic acid per mol of fatty acid, or 36 gm. per 100 gm. of average dietary fat, and 16 gm. of acetoacetic acid per 100 gm. of protein in the diet. The several ketogenic amino acid units of proteins are listed with other *ketogenic substances* in Table 40.

Antiketogenesis

It has previously been indicated that ketogenesis is related to carbohydrate depletion, or disturbance of carbohydrate utilization. When carbohydrate metabolism is readjusted by the administration of glucose and insulin, the accumulated acetoacetic acid disappears. The presence of 1 mol of glucose accelerates the *in vitro* oxidation of 2 mols of acetoacetic acid by hydrogen peroxide. This corresponds roughly to 1 gm. of glucose per 3 gm. of fatty acid. Glycerol is one half as effective as glucose, and the glycerol unit of lipides is quantitatively converted into glucose in phlorrhizinized animals. Hence, 1 mol of a triglyceride should require, in addition to its glycerol unit, only 1 mol of glucose for complete combustion *in vivo*. Details of the metabolism of the antiketogenic glycerol and of the C_3 fatty acid fragment, formed from fatty acids which have uneven numbers of carbon atoms, are given in Chapter V.

The *antiketogenic substances* include glucose, glycerol, and the gluconeogenic carbohydrates and amino acids (Table 40). Glucose is the most antiketogenic sugar; fructose is intermediate in action; and other monosaccharides are less effective. Shaffer's theoretical formulation of the *ketogenic-antiketogenic ratio* of foods is as follows:

$$\frac{K}{A} = \frac{2.4 P + 3.43 F}{3.2 P + 0.57 F + 5.56 G}$$

where F, G, and P are the grams of fat, glucose, and protein in the diet. Numerical values in the numerator represent millimol equivalents of acetoacetic acid, and those in the denominator denote millimol equivalents

TABLE 40

ANTI-KETOGENIC, GLUCONEOGENIC, GLUCOPLASTIC, OR KETOLYTIC SUBSTANCES

CARBOHYDRATES AND RELATED SUBSTANCES		FATTY ACIDS
<i>Dihydroxyacetone</i>	<i>Citric acid</i>	<i>Normal acids with odd number of carbon atoms</i> ¹
<i>Fructose</i>	<i>Fumaric acid</i>	<i>Isobutyl alcohol</i>
<i>Galactose</i>	<i>Gluconic acid</i>	<i>Isobutyric acid</i>
<i>Glycerol</i>	<i>Glyceric acid</i>	<i>Isocaproic acid</i>
<i>Glycerose</i>	<i>Hydroxypyruvic acid</i>	
<i>Glycogen</i>	<i>Lactic acid</i>	AMINO ACIDS ^{2,3}
<i>Glycolaldehyde</i>	<i>Malic acid</i>	<i>Alanine</i>
<i>Maltose</i>	<i>Oxaloacetic acid</i>	<i>Arginine (ornithine)</i>
<i>Mannitol</i>	<i>Pyruvic acid</i>	<i>Aspartic acid</i>
<i>Mannose</i>	<i>Succinic acid</i>	<i>Cystine (cysteine)</i>
<i>Methylglyoxal</i>	<i>Tartaric acid</i>	<i>Glutamic acid</i>
<i>Propyl aldehyde</i>		<i>Glycine</i>
<i>d-Sorbitol</i>		<i>Histidine</i>
<i>l-Sorbose</i>		<i>Hydroxyglutamic acid</i>
<i>l-Xyloketose</i>		<i>Proline</i>
		<i>Serine</i>
		<i>Threonine</i>
		<i>Valine</i>

KETOGENIC OR KETOPLASTIC SUBSTANCES

AMINO ACIDS AND RELATED SUBSTANCES ¹		FATTY ACIDS
<i>2,5-Dihydroxyphenylalanine</i>	<i>Isoleucine</i>	<i>Normal acids with even number of carbon atoms</i>
<i>2,5-Dihydroxyphenyllactic acid</i>	<i>Leucine</i>	<i>Isovaleric acid</i>
<i>2,5-Dihydroxyphenylpyruvic acid</i>	<i>Phenyllactic acid</i>	<i>a-Methylbutyric acid</i>
<i>Homogentisic acid</i>	<i>Phenylalanine</i>	
<i>p-Hydroxyphenyllactic acid</i>	<i>Phenylpyruvic acid</i>	
<i>p-Hydroxyphenylpropionic acid</i>	<i>Tyrosine</i>	
<i>p-Hydroxyphenylpyruvic acid</i>		

¹ Via propionic or pyruvic acid.

² Hydroxyproline and norleucine are considered to be, potentially, both gluconeogenic and ketogenic.

³ Methionine is potentially gluconeogenic (page 427).

of glucose per gram of food. It was formerly supposed that these quantitative relations applied to the general utilization of carbohydrates and fats in the tissues. However, the fact that the liver is largely responsible for ketosis necessitates re-examination of the postulate. A decrease of liver glycogen is the factor which probably initiates ketogenesis, and measures which restore hepatic glycogen are antiketogenic rather than ketolytic. In other words, carbohydrate is a preferential food for the liver, and its utilization suppresses acetone body formation.

Clinical studies have shown that 1 gm. glucose usually allows the oxi-

dition of 1.5 gm. fatty acid without acetonuria. This is only one half the antiketogenic value assigned to glucose by Shaffer. However, only small quantities of acetoacetic acid appear until the ratio is doubled; then severe ketosis occurs. Woodyatt's formula, based on clinical experience, is as follows:

$$G \text{ (Potential Glucose)} = C \text{ (gm. Carbohydrate)} + 0.58 \times P \text{ (gm. Protein)} + 0.1 \times F \text{ (gm. Fat)}$$

$$FA \text{ (Potential Fatty Acid)} = 0.46 P + 0.9 F$$

$$\text{Therefore, } \frac{FA}{G} = \frac{0.46 P + 0.9 F}{C + 0.58 P + 0.1 F} = 1.5$$

Simplification of the equation gives $F = 2 C + 0.54 P$, or, roughly, $F = 2 C + \frac{1}{2} P$. By this simple formula, a diet can easily be appraised for its approximate ketogenic power. If the fat exceeds $2 C + \frac{1}{2} P$, in grams, the diet tends to be ketogenic. The actual threshold of ketogenesis varies with individuals; women and children are more susceptible than men. A diet having a 1.5 ratio can be calculated quickly by the following formulae:

$$\text{Protein} = \frac{2}{3} \text{ gm. per kg. body weight for adults (or 1.5 gm. per kg., for children)}$$

$$\text{Gm. Carbohydrate} = \frac{\text{Calories Required} - (8.9 \times \text{gm. Protein})}{22}$$

$$\text{Fat} = 2 C + \frac{1}{2} P$$

In the modern treatment of diabetes, carbohydrate in excess of Woodyatt's ratio is given.

Dogs, cats, and birds tolerate much higher K/A ratios than do either rats or primates; they dispose of large amounts of injected acetoacetic acid, and do not readily develop ketosis. Humans and certain animals can adapt themselves to high fat diets. In rats, this adaptation can be prevented by incorporating 6 per cent of sodium bicarbonate into the diet. Despite adaptations, the ability of humans to oxidize fat is generally limited and is easily exceeded. Re-establishment of adequate carbohydrate combustion usually corrects ketosis.

In fasting rats or rabbits, injection of anterior pituitary extracts produces ketosis and fatty infiltration of the liver. Removal of the liver, adrenalectomy, or injection of insulin inhibits this ketosis, which is, therefore, referable to a disturbance of hepatic carbohydrate metabolism. (See ketogenic activities, page 690.) Adrenalectomy inhibits the utilization of injected β -hydroxybutyrate in rats.

Interconversions

Certain bacteria produce butyric acid and other short-chain fatty acids from carbohydrate; mammals can perform similar fat syntheses, as shown

many years ago by Lawes and Gilbert. The body fat of pigs (litter-mates) was determined before and after feeding a high carbohydrate diet of known composition. Two months of this feeding produced 18 pounds more fat than could possibly have been provided by the carbon of the limited protein and fat intake. Similar experiments with dairy cattle have shown that these animals can synthesize, daily, more than 250 gm. of butter fat from carbohydrate. Hibernating animals synthesize fat actively during the summer season. In man, conversion of carbohydrate to fat occurs during periods of overeating. Experiments with deuterio labeled foods show that mice synthesize fat from carbohydrate even when the dietary intake is not excessive; they subsequently withdraw the fat from the storage depots and oxidize it without gain in weight. Animals which are given heavy water form some deuterio fatty acids. The deuterium is transferred from the water during reduction of the fatty acid intermediates synthesized from carbohydrate. A high deuterium content of the tissue fatty alcohols suggests that these are intermediates in the synthesis. Other studies with isotopes indicate a condensation of two-carbon intermediates to form the fatty chains. In animals, the glycerol portion of fats is very readily formed from glucose even when insulin is deficient. The conversion of 270 gm. of glucose to 100 gm. of fat, 50 gm. of water, and 115 gm. of carbon dioxide liberates 5 per cent of the carbohydrate calories. A high respiratory quotient results, and relatively saturated, neutral fat is formed. Animals tend to convert carbohydrate to fat which contains palmitic and stearic acids, and the *cis* unsaturated palmitoleic and oleic acids. Insulin and thiamin stimulate fatty acid synthesis; and deficiency of pyridoxin or pantothenic acid interferes with the process in rats. The liver is supposed to be the principal site of the conversion in mammals.

The reverse conversion of fat to carbohydrate can be demonstrated in developing plants and in silkworms. In man, only the glycerol portion of fat is readily convertible to carbohydrate. Neutral fats are, however, easily converted into other lipides by exchange esterification (page 248).

At one time it was believed that adipocere, present in corpses removed from watery graves, represented fat synthesis from protein. Analysis of these cadavers proved, however, that their waxy appearance is largely due to conversion of cadaver fats into insoluble calcium, magnesium, and ammonium soaps of the more saturated fatty acids. Early pathologists believed that fatty degeneration of the liver should be attributed to the conversion of protein to fat; but this has also been disproved by analytical studies. While there is little proof of fat synthesis from proteins in animals, it is known that gluconeogenic amino acids (Table 40) can form carbohydrate; and the latter, together with the few ketogenic amino acids, could, theoretically, form fat under appropriate conditions. However, only slight synthesis has been detected in the many experiments devised to demonstrate such conversions. Protein readily forms carbohydrate, but it is transformed into fat only after the glycogen stores of the body are

filled. To realize this condition requires excess dietary protein and the latter stimulates metabolism so markedly, by its specific dynamic effect, that the net result is little or no fat storage. We may conclude that, in human beings, appreciable quantities of fat are ordinarily synthesized only from carbohydrate foods when the caloric intake is higher than the energy expenditure.

Excretion of Fat

The normal loss of lipides is in the neighborhood of 5 per cent of the intake; it occurs chiefly in the feces, and to a smaller extent in the sebaceous secretions of the skin. Inadequacy of either bile or pancreatic juice causes great loss of fatty acids and fats in the feces. These losses, due, in part, to increased intestinal secretion, cause diarrhea, increased calcium excretion and osteoporosis. An elevated calcium intake increases the fecal lipides (page 153). Only traces of lipide are found in normal urine, but alimentary *lipuria* may result from excessive fat diets. Lipides are excreted in the urine during nephrosis, diabetes mellitus, after fractures of long bones and crushing of bone marrow or subcutaneous adipose tissue, also as the result of cellular degeneration in genito-urinary infections. *Chyluria* occurs when a fistula is established between the biliary and urinary systems.

The mammary gland readily withdraws fat from plasma and converts it to butter fat (Table 32, page 185). The gland apparently utilizes β -hydroxybutyric acid for synthesis of the short-chain fatty acid units of milk fat. The secretion of milk is accompanied by a lipemia that is roughly proportional to the volume of milk secreted. Cattle fed on low fat diets decrease their milk output. The initiation and maintenance of lactation are controlled by the lactogenic hormone of the anterior pituitary gland, the adrenal cortical hormones, and thyroid hormone (page 687).

The lipide secretions of the skin consist of sterols, sterol esters, neutral fats, and a small amount of phospholipide. The neutral fats are found in the *sebum* or oily secretion of the sebaceous glands, while the sterols and sterol esters are derived both from these glands and from the disintegration of cells in the horny layer. *Cerumen* of the outer ear is also a sebaceous secretion. The sebaceous secretions are important for maintaining a normal condition of the skin. The glands of the feet, axillae, and anus secrete more neutral fat than do other sebaceous glands, and rancidity of their fats gives rise to unpleasant perspiration odors or *bromidrosis*. Human skin and hair normally contain about 7 and 4.5 per cent, respectively, of total lipides. The skin lipides are increased by vasodilatation, inflammatory processes, eczema, psoriasis, acne, ichthyosis, and by high fat diets. They exhibit poorly defined relations to the sex hormones. Increased secretion by the sebaceous glands is termed *seborrhea*. The accumulated secretion of the fetal skin is known as the *vernix caseosa*. *Sclerema neonatorum* is a degenerative condition of infancy characterized by deficiency of neutral fat and presence of free fatty acids in the skin.

The external fatty layer of the skin is somewhat hydrous, owing to the sterol and complex lipide fraction. Rancidity and the secretion of lactic acid are responsible for its acid reaction (pH 6). Fat-soluble drugs are absorbed by the skin; they are usually applied as ointments in lanolin or oils. Penetration of water-soluble substances seems to be prevented by the stratum lucidum. It has been reported that rather large quantities of olive oil (100 gm. or more) can be absorbed through human skin during vigorous massage; the absorption occurs mainly through the follicles of the sebaceous glands. The action of ultraviolet rays on the skin produces inflammation, pigmentation, splitting of phospholipides, and important chemical changes in the sterols, such as the synthesis of vitamin D₂ from ergosterol.

PHOSPHOLIPIDES

Digestion and Absorption

Foods contain relatively small quantities of phospholipides; the concentrations in several animal tissues are given in Table 41. Phospholipide concentrations are often reported in terms of phosphorus; such values should be multiplied by 23.5 to convert them to phospholipide concentrations.

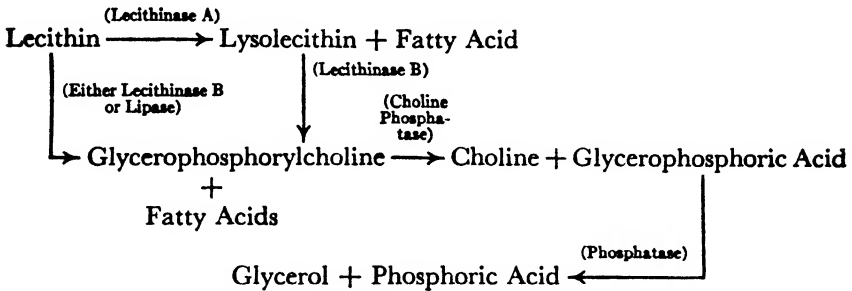
The digestion of phospholipides is quite similar to that of fats, and it occurs principally in the intestine. The enzymes concerned are the lipases, lecithinases, and phosphatases of the pancreatic juice, succus entericus, and bile (Table 27, page 132). The non-specific phosphatases which hydrolyze glycerophosphates (page 597) are secreted in the pancreatic juice and bile, while choline phosphatase is present in the succus entericus

TABLE 41
APPROXIMATE PHOSPHOLIPIDE CONTENT OF TISSUES
(As Lecithin)

	PER CENT
Egg yolk	9.0
Brain	
White	9.0
Gray	4.0
Spinal cord	6.5
Bone marrow ¹	2.8
Kidney, liver	2.5
Adrenal, pancreas, testes	2.0
Heart, lung	1.5
Intestine, muscle, spleen, stomach	1.0
Blood, bones, skin	0.3
Lymph	0.2
Milk	0.02

¹ Higher values in the infant.

The phosphatase activity of the intestinal mucosa increases during fatty acid absorption. The following is a schematic representation of the hydrolysis of lecithins to fatty acids, glycerol, choline, and phosphate:



Following their absorption through the intestinal mucosa, these digestion products participate in fat resynthesis, lymphatic transport, and alimentary lipemia as described in the preceding section.

Blood Phospholipides

The plasma phospholipides of human blood (Table 39, page 215) consist of approximately 60 per cent lecithins, 25 per cent cephalins, and 15 per cent sphingomyelins. The plasma phospholipide level is more constant than the neutral fat level. Increased plasma phospholipide is related to abnormal carbohydrate metabolism. Insulin has a slight tendency to decrease plasma phospholipide, and injection of estrogen in fowls elevates it. The plasma phospholipide concentration rises during alimentary lipemia; most of this extra phospholipide is not of intestinal origin, as shown by experiments with radioactive phosphate. Comparison of results in normal and in hepatectomized dogs indicates that the liver is the source of most of the plasma phospholipide. The erythrocytes contain almost twice as much phospholipide as the plasma; the erythrocyte phospholipide consists of 60 per cent cephalin and 20 per cent each of lecithin and sphingomyelin. Approximately one half the total lipid in leukocytes is phospholipide; the quantity varies considerably with the functional state of the cells. Human blood platelets contain 12 per cent of phospholipide, two thirds of which is cephalin.

When phospholipide, labeled with radioactive phosphorus, is injected intravenously into rabbits, about 50 per cent leaves the blood stream in 1.5 hours. After three hours, one third of the injected phospholipide has entered the liver, and smaller quantities are present in the other tissues.

Tissue Phospholipides

These have been termed the *élément constant* because, in any given tissue, the iodine number and the concentration of the phospholipides are rela-

tively constant even during prolonged starvation or extreme emaciation. A normal storage of phospholipides comparable to that of the neutral fats is not found, although pathological accumulations occur in a number of tissues in Niemann-Pick disease and in amaurotic idiocy. The fetal liver stores considerable phospholipide during the last third of embryonic life. Near the end of pregnancy, the human fetus accumulates daily as much as 50 gm. of total lipide; about two thirds of this is phospholipide. After birth, the tissue phospholipide content decreases rapidly. Phospholipide storage in the liver of a guinea pig embryo can be doubled by injecting phlorhizin into the mother. This procedure also lowers the iodine number of the fetal liver lipides due to mobilization of adipose fat. It has been shown that phospholipides are the source of the phosphate used by chick embryos for ossification and for the synthesis of nucleic acids. In adults, the most readily mobilizable phospholipide is in the liver, and this organ is considered as a temporary depot for transported phospholipides. Ingested phospholipide, labeled with radioactive phosphorus, is retained largely by the liver and the intestine, and is then slowly transferred to other tissues. A small fraction is assimilated by the brain, where it remains for some weeks.

Organs with a high metabolic rate, such as nerve, liver, heart, and kidney, also have a high phospholipide content. The phospholipide content of muscles is largest in those which are most active (heart, diaphragm, etc.). Cholesterol is distributed in similar fashion, and both phospholipide and cholesterol concentrations are greater in malignant than in benign tumors. Actively secreting salivary glands contain more phospholipide than do the resting glands. Phospholipides of the corpus luteum are increased during the most active part of the functional cycle. Hence, the ovarian phospholipide content rises during the first half of pregnancy. The administration of thyroid increases both the quantity and the degree of unsaturation of muscle phospholipides.

Tissue phospholipides are, for the most part, combined with protein and are, therefore, microscopically invisible, unstainable, and are not extractable by ordinary fat solvents although they are soluble in alcohol. The intracellular phospholipides exist partly as potassium salts; this is especially true of the cephalins. It has been suggested that cephalins assist the transfer of base across cell membranes by forming salts in lipide phases and transferring the base to the carbonic acid of aqueous cellular phases. The tissue phospholipides are also important in blood clotting and in immunological reactions (pages 68 and 466).

Cell nuclei contain lecithins, but the sphingomyelins appear to be present only in the cytoplasm. A small fraction of the tissue phospholipides consists of plasmalogens, or acetal phospholipides, in which fatty aldehydes replace the fatty acid units (page 189). Brain and muscle contain 200 and 50 mg. per cent of fatty aldehydes, respectively; the adrenal cortex also has appreciable quantities. The normal liver phospholipides consist of

55 per cent lecithins, 40 per cent cephalins, and less than 5 per cent sphingomyelins. As the liver decreases in weight during starvation, its phospholipide concentration remains constant; the α -lecithins and β -cephalins are removed preferentially during fasting. The normal turnover rates of the hepatic phospholipides apparently decrease in the order: cephalins, sphingomyelins, lecithins. The lecithin fraction rises during fatty infiltration. In addition to the usual fatty acid units, liver phospholipides contain C_{20} fatty acids, and the brain phospholipides have appreciable quantities of C_{22} fatty acids. The lecithin content of the brain is comparable to that of other tissues, but exceptionally large quantities of cephalins and sphingomyelins are present in this organ. The cephalin fraction of human brain lipides increases with the age of the individual. About one third of beef brain cephalin is phosphatidylserine, which also occurs in smaller quantities in other tissues. Much of the brain phospholipide is in the myelin sheaths (Table 41). During pathological demyelinating processes, the nerve phospholipide content decreases. Brain and spinal cord phospholipides contain some dihydrosphingosine. Lung resembles brain in its relatively high sphingomyelin content; the human spinal cord has about 0.7 per cent.

Synthesis

Rats synthesize arsenic-containing analogues of lecithins and sphingomyelins from ingested arsenocholine. Studies with radioactive phosphorus show that tissue slices of liver, kidney, and intestine can synthesize phospholipides from inorganic phosphate. These lipides are continually synthesized in the body, and most actively by the liver and intestine and by certain tumors. Adult brain tissue is least active, although in young animals the central nervous system has a more vigorous phospholipide metabolism. Aerobic oxidation of carbohydrate is coupled with phospholipide synthesis in tissues. Hence, azide, carbon monoxide, cyanide, and sulfhydryl inhibit the synthesis. The sphingomyelins are regenerated more slowly than are lecithins and cephalins; they are synthesized by the liver more rapidly than in muscle. In the liver and intestine, lecithin synthesis is most active, while cephalin is synthesized preferentially in the brain. Administered serine, containing N^{15} , is incorporated into the phosphatidylserine fraction of the phospholipides. Choline, betaine, methionine, and cystine accelerate hepatic phospholipide synthesis, while excess cholesterol inhibits it. Methionine and cystine also prevent hepatic injury from chloroform in protein-depleted dogs. The synthesis of plasma phospholipides is decreased by hepatectomy; the intestine and kidney continue to form phospholipides which are not transferred to the plasma. The phospholipides of egg yolk have been shown to be synthesized in the hen's liver. During incubation of the egg, the yolk phospholipides are mobilized and are altered in the tissues of the embryo.

The degree of saturation of the tissue phospholipides is affected by the

diet. On phospholipide-free rations, chickens synthesize phospholipides which have low iodine numbers. Conversely, the iodine numbers of tissue phospholipides are temporarily raised by fatty meals and especially by the administration of highly unsaturated liver oils. These effects are most pronounced in the liver.

Oxidation

Phospholipides can be readily oxidized in the presence of glutathione and iron salts; lecithins are more reactive than cephalins. The fatty acid units of phospholipides can serve as substrates for fatty acid dehydrogenases. The hypothetical relations of phospholipides to fat oxidation have been considered previously (page 223).

Hydrolysis

The body maintains its *élément constant* by balanced synthesis and hydrolysis. Lysolecithins are found in small quantities in the pancreas, salivary glands, and other tissues. They result from the activity of lecithinase A, which occurs, together with lecithinase B, in many tissues. The hemolytic and cytolytic actions of the lysophospholipides have been mentioned on page 58.

The glycerophosphoric acid fragment of hydrolyzed phospholipides is also an intermediate carbohydrate metabolite (pages 322 and 323). However, injected phospholipides are not converted to carbohydrate in diabetic dogs. Glycerophosphoric acid is rapidly hydrolyzed by tissue phosphatase. Its specific dehydrogenase is especially active in nerve tissue.

Choline phosphatase liberates choline from lecithins and lysolecithins. This enzyme is found in largest amounts in the kidney, intestine, spleen, liver, and pancreas, and in small quantities in many tissues. Choline exists in tissues largely in combined form as phospholipides and acetylcholine. Tissues contain approximately 1 mg. per cent of free choline, and from 40 to 325 mg. per cent of total choline (approximately parallel to the phospholipide concentrations of Table 41, page 230). Seminal fluid contains more than 500 mg. per cent, and blood 35 mg. per cent of total choline. Dietary choline labeled with N^{15} can partly replace that present in phospholipides.

Choline is a lipotropic factor (page 220), and it is a dietary essential when the methionine intake is low. In rats, severe choline deficiency causes fatty degeneration and cirrhosis of the liver, inhibits growth and lactation, and produces marked hemorrhagic degeneration of the kidneys. Tubular degeneration is prominent, together with azotemia, proteinuria, and low inulin and phenol red clearances. In chicks, choline deficiency retards growth and incites perosis (page 598). Studies with N^{15} isotope have shown that the choline radical of lecithins (and sphingomyelins) is synthesized in rats from ethanolamine, and that methionine

and betaine are the methylating agents. Since choline can be synthesized in this fashion, its required intake is inversely proportional to the dietary methionine. Deficiency of methionine, and the administration of cystine or its derivatives, aggravate the hepatic symptoms of choline deficiency, but cystine tends to ameliorate the renal hemorrhage and necrosis. Choline deficiency symptoms are prevented effectively by methionine, betaine, or choline (the important metabolic methyl donors), and by lecithin and phosphocholine. Only choline and manganese salts cure perosis in chicks and turkeys. Choline deficiency decreases the phospholipide turnover and the lecithin concentration in the liver and other mammalian tissues, even though the total choline content of the body may be maintained by continuous synthesis of this substance from ethanolamine.

When free choline is injected intravenously, it is rapidly removed from the blood. Choline can be oxidized to betaine aldehyde and betaine by hepatic enzymes (choline oxidase), which are less active in fatty livers. Some choline is excreted by the liver and the skin; about 7 milligrams of choline are excreted daily in the urine of men.

Experiments with N^{16} isotope show that ethanolamine can replace a part of that present in the cephalins, and can also be converted to choline units of tissue phospholipides in mammals. The ethanolamine unit of the cephalins is apparently formed chiefly by decarboxylation of the amino acid, serine. This reaction is reversible, since serine is produced from administered isotopic ethanolamine or choline. Ethanolamine soaps are very soluble and, when injected, they exert a powerful depressant effect upon blood pressure. Extracts of brain contain a stable and very active depressor substance which is neither histamine, acetylcholine, adenosine, nor adenylic acid; aminoethyl phosphate has been detected in dog brain.

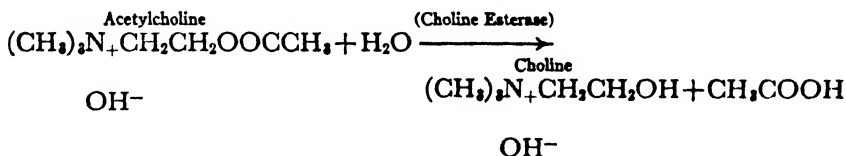
Acetylcholine (Parasympathetic Hormone)

This ester stimulates tissue oxidation and gastro-intestinal peristalsis, and it depresses blood pressure remarkably. It has two general functions as a nerve hormone: (1) a *muscarine action*, or mediation of the peripheral effects of parasympathetic nerves; and (2) a *nicotine action*, or stimulation of autonomic ganglia. Only the first activity is inhibited by atropine. Parasympathetic nerves produce their effects by releasing acetylcholine at the nerve endings. Acetylcholine inhibits embryonic cardiac muscle before it is innervated. The transmission of nerve impulses across ganglionic synapses is accomplished by choline esters. Normal ganglia contain about 1.5 mg. per cent of acetylcholine, which decreases to one tenth this amount after degeneration of the preganglionic fibers. A wave of mobilized potassium ions accompanies the nerve impulse, and increased potassium ion concentration in a ganglion causes liberation of acetylcholine at the synapse, provided calcium ions are also present. Potassium ions liberate acetylcholine in certain tissues which have cholinergic

innervation, and they can excite denervated ganglia without appreciable acetylcholine discharge. Curarine and cobra venom block synaptic transmission by acetylcholine, and inhibit its nicotine action; but these drugs do not prevent the response of ganglia and muscles to potassium ions. Hence, some believe that acetylcholine causes the liberation of potassium ions in muscle. Adrenaline and ephedrine inhibit sympathetic synaptic transmission in a different manner (page 424).

Acetylcholine is liberated at the endings of the preganglionic fibers of the entire autonomic system, the postganglionic parasympathetic fibers, the sympathetic postganglionic fibers of sweat glands, and the voluntary motor fibers. Such fibers are classified as *cholinergic*, in contrast to most sympathetic postganglionic or *adrenergic fibers* which liberate adrenaline-like substances (page 706). Since the innervation of the adrenal gland is cholinergic, acetylcholine is the hormone which stimulates adrenaline secretion. There is evidence that acetylcholine is the active chemical transmitter at the synapses of the central nervous system. Acetylcholine occurs, together with choline esterase and cocarboxylase, along the surfaces of nerve fibers, where it is decomposed and resynthesized during the passage of the impulse.

Ingested acetylcholine is split in the intestine. Only during conditions of circulatory insufficiency do appreciable quantities of acetylcholine appear in the blood. Free acetylcholine cannot exist long in the body, because all tissues (especially the brain, ganglia, nerve sheaths, salivary glands, small intestine, stomach, liver, and muscle tissue in the vicinity of motor nerve endplates) contain a very active enzyme, *choline esterase*. This esterase very rapidly hydrolyzes acetylcholine and the acetyl derivative of thiamin.



The optimum pH of choline esterase is 8.4. The enzyme is activated by calcium, magnesium, and manganese ions, and is inhibited by potassium ions, thiamin, and bile salts. Choline esterase is also inhibited by physostigmine, prostigmine, and morphine, which are, therefore, parasympathomimetic drugs. Denervated muscles show greatly decreased choline esterase activity. The choline esterase found in nerve tissue and erythrocytes is the specific enzyme, while that in other tissues is a variable mixture of choline esterase and pseudocholine esterase. The latter is non-specific, and it hydrolyzes a number of esters. Only the true choline esterase is inhibited by caffeine.

Cholinergic effects are produced by pilocarpine and morphine at the postganglionic nerve endings, and by small doses of nicotine at pregangli-

onic synapses. Since physostigmine inhibits the hydrolysis of acetylcholine by choline esterase, it has been used to detect the hormone in tissues. The largest quantities of acetylcholine are found in peripheral nerve, in the spleen, placenta, and the adrenal gland; the basal ganglia of the brain contain more than either the cerebellum or the cortex.

Acetylcholine may not be the only cholinergic substance of the central nervous system, since an insoluble form of the hormone (a phospholipide derivative?) has been detected in brain. A precursor or inactive combined form of acetylcholine is formed by surviving brain tissue.

If brain slices, perfused ganglia, placenta, or heart muscle extracts are treated with small quantities of physostigmine, they synthesize choline esters *in vitro*. The enzyme concerned, *choline acetylase*, can synthesize the ester anaerobically in the presence of adenosine triphosphate; it is inhibited by α -keto acids and iodoacetate, and is augmented by fluoride. The catabolism of carbohydrate and the presence of thiamin are necessary to replenish the adenosine triphosphate *in vivo*. The acetylcholine content of the cerebral cortex is, therefore, lowered by anoxia or hypoglycemia. Potassium and calcium ions assist the synthesis by brain slices in the presence of bicarbonate, but not when phosphate is used. Since the addition of sodium acetoacetate increases the yield of acetylcholine, it may be a physiological source of the acetyl radical, although acetylphosphate is probably the direct acetylating agent since the oxidation of pyruvate is essential for the formation of acetylcholine in tissues.

A small quantity of acetylcholine is present in the sweat, the largest amounts being secreted during menstruation. The injection of estrone increases the acetylcholine content of the uterus.

CEREBROSIDES

Little is known concerning the metabolism of the cerebrosides. When fed to children, these substances are reported to be largely excreted in the feces. Ingestion of cerebroside by dogs causes the appearance of sphingosine in the urine. Subcutaneously injected sphingosine is also excreted in the urine. Cerebroside emulsions, injected parenterally, seem to be absorbed and utilized. Plasma, erythrocytes, and leukocytes contain a trace, 35, and 210 mg. per cent of cerebroside, respectively. Brain and spinal cord contain about 1 to 2 per cent of cerebroside; nerves and adrenals also contain significant amounts, while other tissues and blood plasma normally have small concentrations. The central nervous system has a higher content of gangliosides than other tissues; the brain ganglioside is increased in amaurotic idiocy. The cerebroside content of brain increases during development. This is especially true of the white matter, where the cerebrosides, cephalins, and sphingomyelins aid in the formation of the myelin sheaths. There is no doubt that cerebrosides are synthesized in the body. The galactose unit of milk sugar is considered important for

the synthesis of normal cerebroside by infants. In at least some cases of Gaucher's disease an abnormal glucose-containing cerebroside is synthesized. This may indicate an anomaly of lipid metabolism.

TABLE 42

APPROXIMATE TOTAL STEROL CONTENT OF FOODS AND TISSUES

FOODS	MG. PER CENT
Egg yolk	1750
Cod liver oil	500
Egg	360
Peanut oil	250
Lard	225
Oysters	215
Butter	150
Olive oil	120
Corn	100
Sardines	90
Cream cheese	85
Cocoanut oil	80
Lard substitute, tallow	75
Chocolate	55
Cream, pork	40
Cauliflower, salmon	25
Bread, green beans, melons, olives, oranges, pears	15
Milk	12
Other common vegetables and fruits	0-10
Egg white	0
TISSUES	MG. PER CENT
Adipose tissue	170
Adrenal	5000
Bile, hepatic	100
Blood	210
Brain	
White	4000
Gray	1000
Kidney	430
Liver	350
Lung	375
Milk	12
Muscle	
Smooth	150
Striated	60
Pancreas	310
Skin	1400
Spleen	250

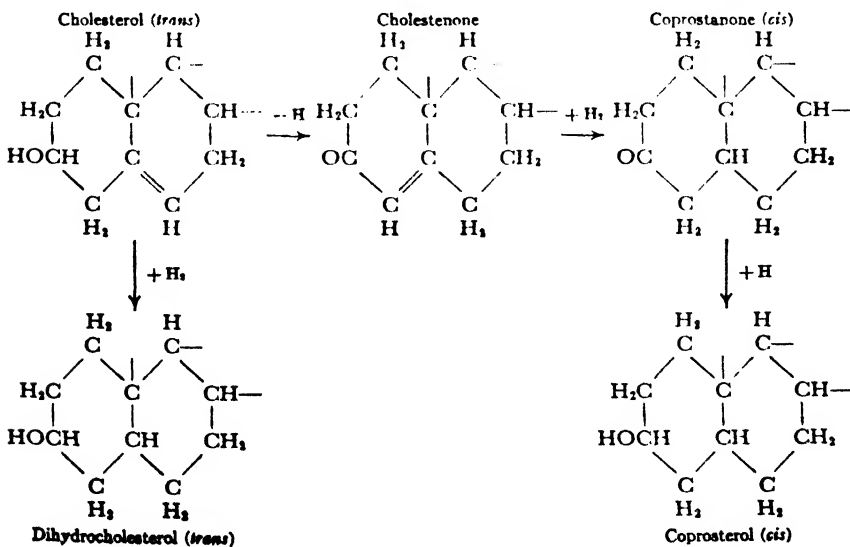
STERIDES

Digestion and Absorption

The human adult daily ingests about 0.33 gm. of cholesterol, obtained chiefly from egg yolk, meat fats, brain, liver, and liver oils (Table 42), and also phytosterols from plant oils, nuts, and seeds. Foods contain varying proportions of free and esterified sterol; the latter is hydrolyzed to free sterol and fatty acid, by pancreatic and intestinal cholesterases, prior to absorption. Normally, the digestive tract of man absorbs about two thirds of the total dietary cholesterol; more than 90 per cent of the phytosterols are lost in the feces. Coprosterol, sitosterol, and sterol esters are not absorbed. Slight absorption of stigmasterol, ergosterol, and dihydrocholesterol has been reported in animals; vitamin D is absorbed more easily.

Efficient intestinal absorption of cholesterol, vitamin D, and other sterides requires the presence of bile salts and unsaturated fatty acids. The sterols are partly re-esterified in the intestinal mucosa during absorption. Pancreatic cholesterase can esterify cholesterol, dihydrocholesterol, and certain sex hormones much more rapidly than the phytosterols; such esterase activity doubtless accounts for the selective absorption of sterides.

About 1 gm. of sterols, chiefly coprosterol, dihydrocholesterol, and cholesterol, is excreted daily in human feces. Coprosterol usually constitutes the largest fraction of the fecal sterols. It is generally regarded as a product of the reducing action of bacteria on cholesterol or its intermediate metabolites, although some coprosterol is apparently secreted by isolated loops of the large intestine. Dihydrocholesterol has definitely been shown to be a secretory product of the small intestine. Experiments with deuterio sterides suggest the following reactions for the production of the fecal sterols (only rings I and II are shown):



Administered cholestenone increases the fecal coprosterol in animals fed with meat, but it increases the fecal dihydrocholesterol when meat is withheld. These results may be correlated with the activity of the intestinal flora (page 154).

Transportation

The mixture of sterols and resynthesized sterol esters is transported by the lymph from the intestine to the blood stream. Normal values for blood sterols are given in Table 39, page 215. The use of oxalated plasma for the determination of cholesterol gives low values and should, therefore, be avoided. The cholesterol esters of blood plasma include the oleate, linoleate, palmitate, and stearate. The esterified cholesterol of blood varies much more than the free cholesterol; the plasma contains a cholesteraase which may affect the distribution. The total cholesterol of plasma rises only slightly after the ingestion of cholesterol and fats. Some have interpreted this cholesteroemia as an indication of the participation of plasma cholesterol esters in the transport of fatty acid units. The liver assists actively in the regulation of blood sterol ester levels. Damage to the liver parenchyma, as in hepatic disease or biliary obstruction, may cause the plasma cholesterol esters to fall to very low values. Thyroglobulin tends to shift cholesterol from the blood to the tissues. Human plasma cholesterol is frequently increased during the lipemia of pregnancy, and it tends to be lower following menstruation. Fetal venous blood contains less than half as much cholesterol as the maternal blood; the level rapidly increases immediately after birth.

Only free cholesterol, which is antihemolytic, is present in the erythrocytes. The leukocytes contain considerably more cholesterol than do erythrocytes. Normal cerebrospinal fluid and transudates contain only traces of cholesterol. Injection of deuteriocholesterol shows that the lungs and liver remove it from the blood readily, but the brain does not.

Tissue Sterols

Sterols are essential constituents of plant and animal cells. Many bacterial cells contain only traces of sterols. The human body has approximately 50 gm. sterol, almost one half of which is in the brain. This sterol mixture consists of approximately 98 per cent cholesterol and its esters, 2 per cent dihydrocholesterol and traces of ergosterol, cholestenone, 7-dehydrocholesterol, and steride hormones. At term, the human fetus assimilates approximately 7 gm. of free cholesterol and 8.5 gm. of cholesterol esters daily. The cholesterol content of the brain increases rapidly in the growing fetus, infant, and child; the infant deposits about 25 mg. brain cholesterol daily. The proportion of ergosterol rises until birth and then falls, temporarily, during infancy and early childhood when active ossification is in progress. The adult brain contains approximately from 2 to 3 per cent cholesterol, present chiefly in the white matter; this tissue

and the adrenal have higher cholesterol concentrations than other tissues (Table 42). The cholesterol of the adrenal gland decreases after carbohydrate administration or injection of corticotropic hormone.

The brain, erythrocytes, heart muscle, and bile contain free cholesterol and only traces of esters, while other tissues have significant amounts of the cholesterol esters. The latter do not appear in the adrenal cortex of normal infants until the third month of life, but they are found at birth in the adrenals of anencephalic monsters. Later in life, the adrenal glands contain large proportions of cholesterol esters. Adrenalectomy raises the cholesterol esters of the blood serum, while the amount in the liver, brain, and muscles is decreased. The administration of adrenal cortical hormone has an opposite effect. Cholesterol esters increase in the chick embryo during the last third of embryonic life, at the time when fatty acid and glycerophosphate units are freed from phospholipides for osseous calcification. In the human embryonic liver the esters appear by the end of the first month, increase to the fifth month and then disappear. The liver and the intestine are regarded as the chief organs in which sterol esters are formed.

In muscle, the cholesterol and phospholipide concentration is proportional to the functional activity. These lipides tend to counteract each other in tissue metabolism (pages 53 and 58). The *lipocytic coefficient*, expressed as

$$\frac{\text{lipide P}}{\text{total cholesterol}} \times 100,$$

tends to remain constant in animals.

Storage

When excessive quantities of cholesterol are incorporated in the diets of herbivorous animals, hypercholesterolemia occurs. Cholesterol esters and cholesterol are then deposited in most organs except the brain, and atherosclerosis develops. Carnivora differ from herbivora in this respect. Their livers excrete cholesterol rapidly and thus prevent massive cholesterolemia and subsequent infiltration. The poorly absorbed phytosterols cause neither sterolemia nor atherosclerosis, even after prolonged feeding to rabbits. Normally, the body stores cholesterol in the form of esters. These esters are not deposited in adipose tissue but appear as mobile deposits, especially in the liver, the adrenal cortex, and the corpus luteum of the ovaries. The esters of the adrenal cortex are chiefly the palmitate and the stearate. Cholesterol ester storage has some relation to cholesterol intake, but it is largely subject to unknown endogenous factors. The lipotropic factors which affect hepatic cholesterol ester deposition are considered on page 219. Castration, or the ingestion of bile, increases the deposition from high cholesterol diets. Certain pathological processes which cause abnormal deposition of cholesterol esters in arteries, kidneys,

and other tissues are discussed on page 254. Leukocytes become infiltrated with the esters during infections and after operations. Tissues with poor capillary supply, such as the aorta, lens of the eye, cornea, costal cartilage, and tympanic membrane are especially subject to sterol ester infiltration. Even the brain can be the site of ester deposits during disease. These pathological depositions are much more permanent than the normal labile deposits. It has long been recognized that the microscopically visible deposits of cholesterol esters in diseased and aging tissues are signs of slow cellular degeneration.

Synthesis

Since plant sterols are absorbed very poorly, they cannot be considered as cholesterol precursors in animals. Food cholesterol is not the only source of cholesterol in the animal body, as proved by balance experiments. During the first month of life, dogs on low cholesterol diets can increase their body cholesterol from twenty to thirty times the amount present in the food. Synthesis has also been demonstrated in infants, and in rats and other animals. The lack of cholesterol in the normal diets of herbivorous animals also indicates extensive synthesis. The markedly increased cholesterol and cholesterol esters in the muscles of rabbits suffering from nutritional muscular dystrophy are synthetic in origin. On low cholesterol diets, infants excrete sterol in excess of the intake. Chicks excrete cholesterol rapidly after hatching, and additional amounts are destroyed in their bodies; following this early loss, synthesis is demonstrable.

It has been estimated that the human synthesizes about 4 gm. of total sterides daily, including cholesterol, bile acids, sex hormones, and so forth. Cholesterol is very probably formed from glucose; molds can make it from this sugar. Animals which are given heavy water synthesize cholesterol containing 50 atoms per cent of deuterium. This is analogous to the synthesis of fatty acids from carbohydrate, where every second hydrogen atom is derived from the water of the body. Deuteroacetate, and other deutero compounds which form acetyl radicals in animals, can be used for the synthesis of cholesterol. Their deuterium appears in both the nucleus and the side chain of tissue cholesterol, which may therefore be constructed from two-carbon residues. Regeneration of one half of the body cholesterol requires twenty days, three times the period required for fatty acid regeneration. The intestine and liver are apparently the most active cholesterol-synthesizing tissues. When squalene is fed to rats, the cholesterol esters of the liver are increased. However, squalene is most important in selachian metabolism; it seems to be replaced in human livers by other highly unsaturated hydrocarbons. Pathological accumulation of squalene occurs in dermoid cysts of the ovary. Experiments with deutero-hexadecane show that it is absorbed from the rat's intestine, is deposited in tissue lipides, and is partially oxidized to fatty acids. Certain sterides and fat-soluble hydrocarbon derivatives cannot be synthesized in sufficient

quantities by man, as evidenced by the indispensability of vitamin A. (See pages 645 to 651 for carotene metabolism.)

Oxidation and Degradation

Normal tissues exhibit balanced synthesis and destruction of cholesterol, and the tissue sterol content tends to remain constant. Large quantities of cholesterol can be destroyed in animals, but the reactions involved are not known. A small portion is reduced to dihydrocholesterol; another minor fraction is oxidized to a product which resembles cholestenone. The liver may form bile acids from cholesterol oxidation products. By oxidation to cholestenone and subsequent reduction, *trans* cholesterol can be converted into *cis* derivatives of coprostane.

Balance experiments show that considerable absorbed cholesterol must be destroyed in mammals. When phytosterols are injected intravenously, they are partly excreted in the feces and partly destroyed. Hence, there is only slight storage of ingested ergosterol.

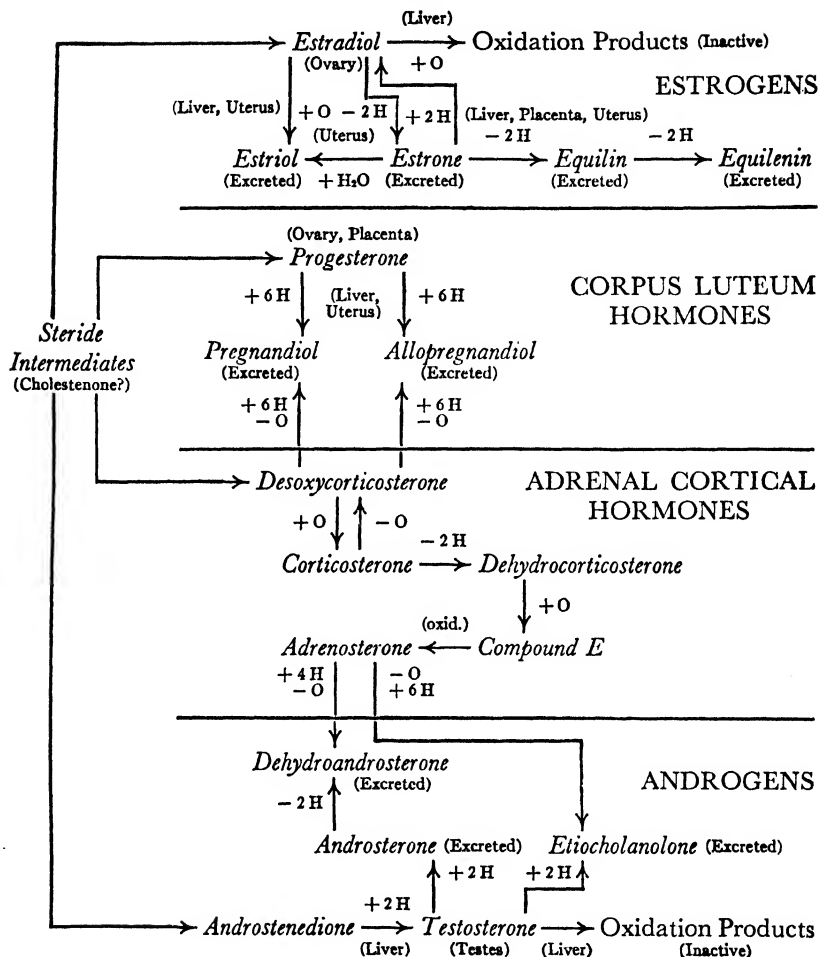
Sex Hormones and Adrenal Cortical Hormones

The formation of sex hormones from cholesterol would require degradation of the side chain, perhaps through oxidation. When deuteriocholesterol is administered orally to pregnant women, some of the deuterium is found in the urinary pregnandiol glycuronide. Under regulation of the gonadotropic hormones of the anterior pituitary gland, estradiol and progesterone are formed in the ovary, and testosterone in the testis. The formation of desoxycorticosterone, corticosterone, and related hormones in the adrenal cortex is stimulated by the corticotropic pituitary hormone. Estradiol, progesterone, corticosterone, desoxycorticosterone, and testosterone are produced from unknown steride intermediates; they can be transformed by oxidation, reduction, and rearrangement, as shown in the diagram on page 244.

The end products of sex hormone metabolism are excreted in the urine, partly as glycuronides. When estrogens are administered to human beings more than 80 per cent is converted to inactive oxidation products. Injected adrenal cortical hormones are also inactivated rapidly in the body. Details of the activities and metabolism of the steride hormones are given in Chapter X.

Excretion

Only a trace (0.5 mg. daily) of cholesterol is found in normal urine. Because of its lipid solubility, cholesterol is excreted principally by the intestine, liver, and skin. Approximately 1 gm. of free cholesterol is secreted daily in human bile; but much of this is later reabsorbed, with the bile salts, in the small intestine. Biliary cholesterol output is independent of plasma cholesterol levels. It increases in late pregnancy, but



is not affected by the hypercholesterolemias of diabetes and nephrosis. It is lowered during severe hepatic disease or biliary obstruction. The small intestine also excretes dihydrocholesterol, as proved by the accumulation of this substance in isolated intestinal loops. Coprosterol does not seem to be excreted appreciably as such, for it is absent from the feces of infants and of adults maintained on milk diets. The daily quantity of human feces contains about 1 gm. of total sterol; 100 to 150 mg. are excreted daily by the skin. The skin cholesterol is increased in the parakeratosis (ichthyosis, psoriasis), chronic eczemas, neurodermatitis, essential pruritus, vitiligo, and onychomycosis; it tends to be low in acne, furunculosis, and alopecia areata. The water-soluble esters and glycuronides of the sex hormones are excreted in the urine.

Vitamin D

The vitamins D of animal fats and oils represent a mixture of closely related steride derivatives. The most important component is the vitamin D₃, isolated from tuna fish liver oil. Irradiation of the skin with ultra-violet light causes the synthesis of vitamin D₃ from 7-dehydrocholesterol. Vitamin D₂ (calciferol) is similarly formed from ergosterol; the latter constitutes 0.5 per cent of the skin sterols. Certain mammals lick their fur, and thus ingest vitamin D. Scrubbing the skin frequently and thoroughly with soap and water is harmful to herbivorous animals, for it removes their chief dietary supply of the vitamin. Fish synthesize relatively large quantities of vitamin D without the necessity of irradiation; their liver oils form an important clinical source of the vitamins D. After absorption through the intestine or from ointments applied to the skin, these vitamins are transported to the tissues. In areas of active ossification, they regulate calcification processes (page 590). They also increase the absorption of calcium in the intestine, thus making it available for ossification. The comparatively low ergosterol content of the brain and skin of infants may indicate active utilization of this sterol for the production of vitamin D₂. Since mammalian liver oils contain much less vitamin D than do the fish liver oils (even after liberal administration of the vitamin), it is evident that vitamin D is easily destroyed in mammals (page 668).

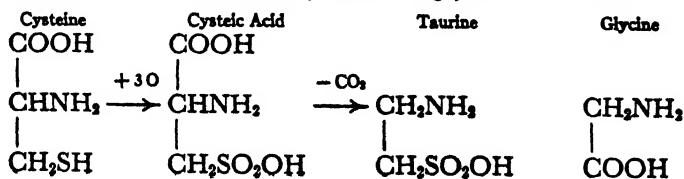
Bile Acids

These sterides constitute about 2 per cent of human hepatic bile. Their role in absorption and their enterohepatic circulation have been discussed on pages 149 and 143. Continued loss of bile from an external biliary fistula is eventually fatal. Dogs with such fistulae secrete 1 gm. or more of cholic acid daily (0.115 mg. bile acid per kg. body weight), an amount which exceeds their sterol intake. Mammals can, therefore, synthesize bile acids from endogenous precursors. When bile is re-fed to these animals the daily biliary secretion of bile salt increases sevenfold, owing to recirculation. Analogous findings in human patients are: approximately 1.5 gm. bile acid per day, which is tripled when the bile is re-fed. Bile acid secretion is increased in bile-fistula dogs by a protein diet, but not by fat or carbohydrate feeding or by injecting cholesterol. However, injected deuterio-cholesterol is converted to cholic acid in dogs. Since the bile acids have *cis* configurations, their formation from *trans* cholesterol would require a stereochemical inversion. This reaction may be accomplished by the oxidation of cholesterol to intermediate unsaturated derivatives, such as cholestenone or ketocholenic acids. The latter are present in small quantities in bile. Bile acid production, by whatever path, is a specific function of the vertebrate liver. When the common bile duct is obstructed completely for more than one week, bile salts disappear from the hepatic bile

and definite hepatic lesions result. The bile salts reappear several weeks after relief of the obstruction. Low concentrations of bile salts in drainage bile indicate severe damage to hepatic cells, and decreased bile acid synthesis.

The liver readily assimilates bile salts from the blood stream. During their enterohepatic circulation, about 90 per cent of the bile acids are normally reabsorbed through the intestine. Normal polygonal cells remove bile salts rapidly and almost completely from the blood. Systemic blood contains only very minute concentrations of bile salts (1.5 mg. per cent). Portal blood contains somewhat larger concentrations, namely, 2 to 5 mg. per cent. Normal urine contains mere traces of bile salts, but the intravenous injection of these salts produces *choloria* or the excretion of bile acids in the urine. However, the major fraction of the injected bile salt is removed rapidly by the liver and is excreted in the bile within a few hours. The biliary volume increases, since the biliary bile salt concentration is relatively constant. Injected bile salts cause bradycardia, lowered blood pressure, and lesions of the renal tubules. When given by mouth, they have a cholagog effect, and they produce catharsis by stimulating the musculature of the large intestine. The intravenous injection of from 0.1 to 0.4 gm. bile salt per kg. of body weight is fatal to animals; approximately ten times as much is required by subcutaneous injection. Dehydrocholate is least toxic, desoxycholate most toxic, and the conjugated bile salts are intermediate. Bile salts lyse erythrocytes, pneumococci, and so forth; this effect, and the bradycardic action of bile salts, are partially inhibited by plasma proteins, which adsorb the bile acids. Recent experiments indicate that one half the bile salts formed daily are destroyed in the liver. Cholic acid is a "gizzard factor" for chicks; it cures lesions of the gizzard caused by nutritional deficiency or by cinchophen administration.

Conjugation of Bile Acids. The bile salts in normal human bile are largely conjugated, 80 per cent as glycocholates and 20 per cent as taurocholates. The distribution of the bile acid components is approximately 60 per cent cholic acid, 20 per cent desoxycholic acid, and 20 per cent chenodesoxycholic acid. Conjugation of bile acids by the liver resembles the detoxication of benzoic acid. The amino acids most commonly used by the human body for *detoxication* are those found in glutathione, namely, glycine, cystine (or cysteine), and glutamic acid (page 430). While glycine is used directly to produce glycocholates, cysteine is first oxidized and decarboxylated in the liver and kidney to taurine, which is then conjugated with the bile acids. Note the similarity between glycine and taurine:



The liver is an especially active conjugating organ. Hepatic disease diminishes the conjugation of glycine with administered benzoic acid and also with bile acids. When colic acid is fed, it increases the biliary excretion of taurocholates until the taurine precursors of the body are exhausted. Subsequent administration of methionine, cystine, cysteine, homocysteine, cysteic acid, or taurine maintains the taurocholate output. Taurine precursors are exhausted by starvation, but normal diets provide quantities in excess of the body's needs. When bromobenzene is fed to cats, it is preferentially detoxicated by taurine precursors and the biliary taurocholates decrease. The human liver conjugates cholic acid most easily with taurine. Hence taurocholates preponderate whenever biliary secretion is diminished, as after operation for the relief of obstruction. Conversely, the normal copious secretion of bile favors conjugation with glycine. The liver and kidney contain enzymes (cholases) which can hydrolyze the conjugated bile acids.

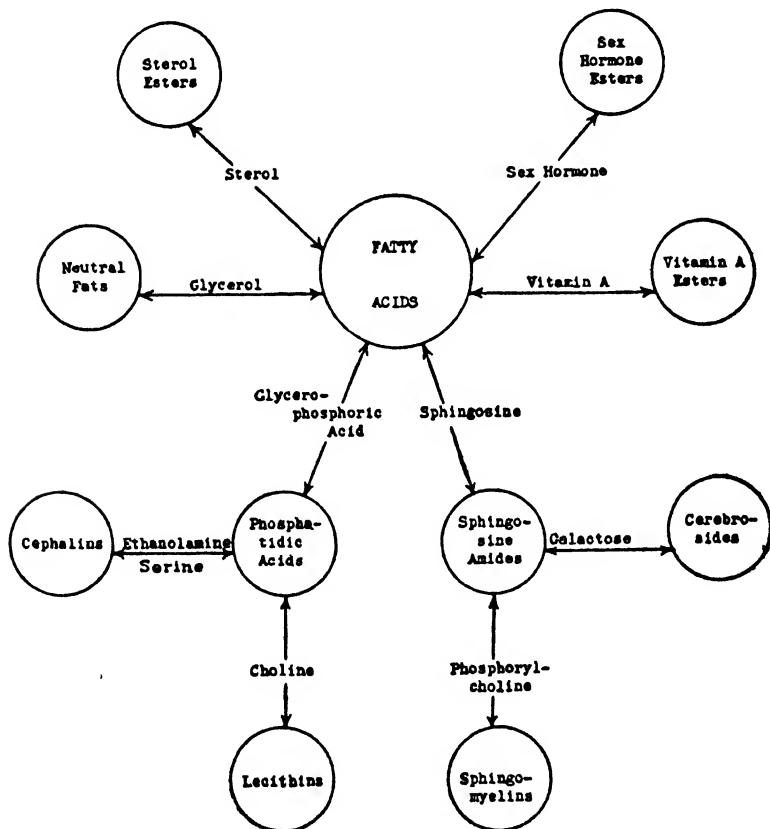


FIG. 4. Exchange esterification of lipides.

EXCHANGE ESTERIFICATION OF LIPIDES

The interrelations of lipides through exchange esterification are visualized in Figure 4. The fundamental fatty acid units of lipides are readily transferred, by esterification reactions, from one lipide to another. At the circumference of the diagram, the lipides have been placed in physiologically related order. Examples of serious imbalance of exchange esterification are given in the following section.

PATHOLOGY

"Where the conclusion seems excellent we are not critical as to the supporting arguments." — MORRIS R. COHEN

LIPEMIAS

Lipemias generally indicate mobilization of neutral fats, although the phospholipides and sterols of plasma frequently participate in the lipide increase. Lipemia appears temporarily after high fat meals, or after severe hemorrhage; but it becomes a more permanent condition during chronic alcoholism, various anemias, anesthesia, diabetes, fever, hypothyroidism, obstructive jaundice, leukemia, liver infiltration, nephrosis, chronic glomerulonephritis, pregnancy, and starvation. Some of these lipemias do not require the absorption of dietary fat for their development, although they are aggravated by high fat diets. Diabetic and nephrotic lipemias, at times, reach very high levels. The former is often associated with ketosis and degenerative phenomena, and it is rapidly reduced by the injection of insulin. During pregnancy, the plasma neutral fat gradually increases until term, at which time the level may be twice normal. All of the plasma lipides, except the cholesterol esters, increase at the onset of fever, and at its height all but the neutral fats fall. Lipemia appears on the second day of complete starvation, continues for about two weeks and then decreases slowly as the depot fats become exhausted.

A low blood fat level, *lipopenia*, is found in hyperthyroidism, chronic hemorrhagic anemias, and prostatic hypertrophy. The determination of fat or fatty acids is used infrequently in the clinic, because of technical difficulties, and because more satisfactory procedures for the determination of plasma cholesterol are available.

Increased plasma phospholipide has been reported in diabetes, chronic hemorrhage, lactation, nephrosis, Niemann-Pick disease, pregnancy, and xanthomatosis. The plasma phospholipide is subnormal in pernicious anemia during relapse; it rises again at the onset of remission. It is also low in acute fevers and in hyperthyroidism. The iodine number of the plasma phospholipides is reported low in children with eczema or acute infections. In anemias and hyperthyroidism, the plasma lipide iodine number is high. The phospholipide of leukocytes falls during infections,

and rises during convalescence. The cerebroside content of the erythrocytes increases in pernicious anemia and diabetic lipemia.

STARVATION

The mobile carbohydrate stores of the human body become exhausted by the second or third day of complete starvation, and subsequently energy is derived chiefly from the oxidation of stored fat and of tissue protein. The quantity of stored fat largely determines the time of survival of a starving animal. Exhaustion of the fat depots initiates rapid oxidation of protein derived from the least essential tissues. Approximate tissue losses in the cat during prolonged starvation are given in Table 43. The fat depots decrease most rapidly during the first few days, during which time large quantities of extracellular water are also lost. The brain, heart, and other essential tissues are maintained almost until death. As body weight decreases, the basal metabolism gradually diminishes, together with the pulse rate, blood pressure, and body temperature. Meanwhile, carbo-

TABLE 43
APPROXIMATE WEIGHT LOSSES DURING
PROLONGED STARVATION

	PER CENT LOSS
Adipose depots	95
Spleen	70
Liver	50
Genital organs	40
Skeletal muscles	30
Blood, kidneys	25
Intestine, lungs, pancreas, skin	20
Bone	15
Brain, cord, eyes, heart	5

hydrate continues to be formed from protein in amounts sufficient to prevent severe ketosis (page 37). This gluconeogenesis also prevents the development of hypoglycemic shock, and allows the maintenance of limited levels of tissue glycogen in the starving animal.

OBESITY

Obesity is defined as excessive deposition of neutral fat in the storage depots, resulting in a body weight more than 10 per cent above the ideal. This condition should not be confused with edema caused by insufficient protein or low vitamin intake. Obesity is associated with a relative excess in caloric intake and with deficient muscular activity, even when these factors are not the fundamental cause of the condition. Obese patients have abnormal appetites. Slight caloric excess will gradually produce

obesity; an extra teaspoonful of butter, or two teaspoonfuls of sugar, will increase the average daily caloric intake by 3 per cent, and a daily walk of one mile is required to counteract this excess. A daily excess of two thirds of a glass of milk or of one large orange can increase the body weight 10 pounds in a year. By reason of such dietary excesses, obesity occasionally begins during lactation or convalescence.

Clinicians recognize two general types of obesity: (1) *exogenous* or *alimentary obesity*, from overeating; and (2) *endogenous* or *endocrine obesity*, due to metabolic abnormalities. There is no sharp line of demarcation between these types. In exogenous obesity, which is most common in males over thirty years of age, the chief deposits are on the trunk. There are several well recognized subtypes of endogenous obesity with endocrine etiology. Obesity is sometimes hereditary, but certain familial tendencies are undoubtedly referable to habits. Administration of deuterio fatty acids to congenitally obese mice indicates that fat storage is normal in rate, but that the turnover and catabolism of depot fat are retarded.

The central nervous system plays a role in peripheral fat storage; neural atrophy causes a loss of fat in the affected areas. True muscular dystrophies involve atrophy of muscle tissue rather than fat deposits. Nervous influences are supposed to be important in the following types of obesity. A *cerebral type* results from encephalitis, cerebral hemorrhage, and congenital brain abnormalities; in these cases, fat is deposited over the entire body. An interesting *regional type*, which affects only the lower half of the body, is rather common in women; when, in addition, the subcutaneous fat deposits of the upper portion of the body atrophy, the condition is called *lipodystrophy*. Sharply circumscribed atrophy of adipose tissue can also occur; such fatty atrophy results, at times, in subcutaneous tissues from trauma produced by repeated injection therapy, or prolonged diathermy or chilling.

In *hypothyroid* or *thyrogenic obesity*, fat is deposited both on the trunk and on the extremities. The hands are spadelike, the skin is dry, mental retardation is pronounced, and the basal metabolic rate is lower than that predicted from the ideal weight of the patient. Thyrogenic obesity responds to the administration of thyroid. Not over 10 per cent of clinical obesities involve hypothyroidism.

The *hypogonad* or *genital type* of obesity commonly occurs as climacteric obesity in women, although occasionally it appears at an earlier age. Hypogonad obesity is associated with changes in secondary sex characteristics, and with typical apronlike depositions from the waist to the thighs. Castrates develop genital obesity. Increased urinary excretion of gonadotropic substances differentiates hypogonad obesity from hypophyseal types (page 686).

The *hypophyseal types* of obesity are often accompanied by hypogenitalism, infantile facial characteristics, slender extremities, and small hands and feet. The hypophyseal obesities include *dystrophia adiposogenitalis* (Fröh-

lich's syndrome), *Cushing's syndrome*, and *adiposis dolorosa* (Dercum's disease). In the last named condition, painful pendulous fat tumors develop, and there are indications of both thyroid and pituitary involvement. The adrenal cortex is known to have certain relations to fat deposition.

Marked obesity predisposes to arterial hypertension, cardiac complications, circulatory insufficiency, eczema, gallstones, excessive perspiration, and sterility. Obesity in susceptible adults predisposes to diabetes mellitus; it also increases susceptibility to infections and to postoperative hernia. For these reasons, the life insurance rates for obese persons are high.

Treatment of Obesity

In the dietary treatment of obesity it is essential to provide an insufficient caloric intake, to avoid ketosis, to prevent protein and vitamin deficiencies, and to allay the distress of hunger. In planning the dietary regimen, the education of the patient should be kept in mind. The allowable caloric intake is usually from 20 to 35 per cent below the calculated requirement for maintenance of the *ideal* body weight of the patient. Reducing diets, therefore, contain from 800 to 1400 calories per day, depending on the ideal weight, the degree of obesity and complications. Such diets accomplish a weekly loss of approximately from 3 to 5 pounds. After the body weight has been decreased by 20 or 25 pounds, the caloric intake is usually increased just sufficiently to maintain the new weight for a few months and then the process is repeated. Gradual reduction of obesity gives the best results. Much more restricted diets have been popular in untrained hands; they should be avoided because they lead to anemia, weakness, myocardial failure, and other distressing symptoms. Slight obesities (10 to 15 per cent overweight) require dietary regulation only when such complications as heart disease, gout, or diabetes are present.

Certain patients show no loss of weight for days because the fat removed by the reducing diet is balanced by temporary water retention. This edema, due to partial starvation, is abolished by increasing the dietary protein and administering thiamin. Retention of water and subsequent diuresis may lead to stepwise weight loss, making it desirable to weigh the patient weekly rather than daily. Restriction of water is not usually desirable.

The reducing diet should include milk, eggs, lean meat, fish, fruit, clear soups, and vegetables having from 3 to 6 per cent carbohydrate. The choice of foods involves the selection of proteins with high biological value, provision of dietary bulk for maintaining normal motor activities of the intestine, and consideration of the *satiety value* (ability to relieve hunger). Meat proteins and clear meat broths have high satiety values; potatoes are better than bread; and fruits or small portions of dessert are desirable at the end of the meal. The daily dietary protein should

range between 1 and 2 gm. per kg. of body weight in order to prevent edema and loss of tissue protein, and to increase the caloric output by the specific dynamic action. The remainder of the diet consists largely of carbohydrate; a little butter is given since it is a carrier of fat-soluble vitamins. Green vegetables and low calorie salads are important in the diet.

For endogenous obesities, endocrine therapy is instituted when indicated. Thyroid has been found useful in the treatment of hypothyroid obesities. Thyroid therapy requires careful control to avoid excessive basal metabolic rate, increased pulse rate, nervousness, and other thyrotoxic symptoms. Dinitrophenol administration is dangerous since it leads to liver injury, cataract, and other toxic effects. Mild exercise is instituted unless it is contraindicated by heart disease, arteriosclerosis, or other complications. Benzedrine therapy is used to decrease appetite in patients afflicted with anhedonia (a neurosis which sometimes results in excessive eating), but there is danger of habit formation.

HYPERCHOLINERGIC AND HYPOCHOLINERGIC CONDITIONS

Prostigmine, supplemented by guanidine, ephedrine, and potassium chloride, is used therapeutically in treating *myasthenia gravis*, a condition in which there is an elevated neural end plate threshold, muscular weakness, and rapid fatigability. In these patients, muscle choline esterase activity is increased; prostigmine administration temporarily causes diminution of the choline esterase activity of muscle and blood, with restoration of normal muscular function. It is also claimed that prostigmine facilitates recovery from the lower motor neurone paralysis caused by motor nerve section or by intraneural injection of alcohol. In denervation atrophy, quinine prevents fibrillation by decreasing the sensitivity of the muscle to acetylcholine and potassium ions. Administration of quinine aggravates muscular weakness in *myasthenia gravis* by causing further depression of the end plates, but is beneficial in *myotonia congenita*, where there is peripheral hypercholinergic activity, muscular hypertonicity, and slow relaxation. This inherited myotonia is aggravated by prostigmine, physostigmine, or potassium salts. Quinine, adrenaline, or calcium salts relieve the myotonus but cannot improve muscular function in dystrophic myotonia (*myotonia atrophica*), a hereditary degenerative condition with symptoms of emaciation, muscle atrophy, testicular atrophy, and cataract (page 462). Studies of congenital myotonia in goats have shown that the after contractions are due to an asynchronous tetanus of the muscle fibers. The myotonic muscle is abnormally sensitive to potassium cations, which cause a prolonged contraction. In these animals, injection of desoxycorticosterone acetate, calcium chloride, or vitamin D causes temporary disappearance of the myotonia. In severe anemias (other than pernicious anemia) the choline esterase activity is increased in the erythrocytes and lowered in the plasma. The plasma choline esterase

activity is decreased in jaundice, hepatic cirrhosis, cancer, and malnutrition; it is increased in beriberi and hyperthyroidism.

HYPERCHOLESTEROLEMIA

Plasma cholesterol rises during lipemia and hypoproteinemia. Hypercholesterolemia is encountered most frequently in acute hemorrhagic anemia, anesthesia, arteriosclerosis, biliary obstruction, diabetes, hypothyroidism, nephrosis, chronic glomerulonephritis, pregnancy, starvation, and xanthomatosis, and after plasmapheresis. In diabetic patients, the plasma cholesterol may occasionally rise above 1000 mg. per cent; insulin therapy reduces diabetic hypercholesterolemia. The plasma cholesterol level provides a convenient index to diabetic lipemia and to the general condition of adult diabetic patients. It is especially useful as an indication for further therapy after the blood sugar level has been restored to normal. However, plasma cholesterol determinations are not very helpful in the management of diabetes in children. Hypercholesterolemia and an increase in plasma sterol esters is a rather constant finding in nephrosis, where it may be as marked as it is in diabetes. In nephrosis, hypercholesterolemia is associated with deposition of cholesterol esters in the renal tubular epithelium and their subsequent excretion in the urine (cholesteroluria). The cholesteroluria parallels the excretion of plasma protein, and the hypercholesterolemia of nephrosis is reciprocally related to the severity of the edema and the hypoproteinemia. Plasma cholesterol determinations are helpful in the diagnosis and prognosis of hypothyroidism, particularly in infants and children.

Hyperthyroid conditions cause *hypcholesterolemia*, which parallels the increase in basal metabolic rate; dinitrophenol does not have this effect. The blood cholesterol is also subnormal in non-hemorrhagic anemias, hepatic disease, prostatic hypertrophy, and in cachexial and febrile conditions. Hypocholesterolemia is especially marked in hemolytic jaundice and in uremia, as well as during relapse of pernicious anemia. In the latter condition, it is associated with lipemia, low plasma phospholipide, and increased cholesterol ester content of the erythrocytes; normal lipid levels are re-established during remissions. The plasma cholesterol, especially the ester fraction, falls in severe hepatocellular disease. When this occurs in jaundice, it indicates that the mechanical obstruction is complicated by serious degeneration of liver parenchyma. The plasma cholesterol ester level is, therefore, an index to improvement in degenerative hepatic diseases.

Cholesterol appears in small quantities in cerebrospinal fluid during meningitis, cerebral hemorrhage, brain tumors, and brain abscesses. Cholesterol is also present in inflammatory exudates. In chronic exudative processes, repeated aspiration reduces the cholesterol content of the effusion.

SECONDARY LIPIDE DEGENERATIONS AND DEPOSITIONS

The anisotropic or doubly refracting droplets noted in degenerating and necrotizing tissues are deposits of cholesterol esters. They occur in caseous areas, old infarcts, inspissated pus, dermoid and brain cysts, hydrocele fluid, certain tumors, nephrotic kidney tubules, degenerating brain tissue, atheromatous arteries, and so forth. *Atherosclerosis* is a form of arteriosclerosis in which lipides, and especially cholesterol esters, accumulate in the intima of arteries and arterioles. Pseudoxanthoma cells appear, and the arterial intima degenerates with the formation of ulcers or abscesses which may rupture and cause hemorrhage. Necrosis, calcification, and cartilage formation complicate the arterial changes. The tissues of the adrenal, bone marrow, eye, kidney, liver, lung, and spleen may show similar involvement. The aorta is most sensitive to the depositions, which can be produced in herbivorous animals by feeding cholesterol (page 241). In addition to cholesterol, the human arteriosclerotic aorta contains small quantities of dihydrocholesterol, several cholestadienones, cholestanetriol, 7- β -hydroxycholesterol, and batyl alcohol. Atherosclerosis is most common after midlife; it is provoked or aggravated by diabetes, lipemias, and high lipid diets. This is one reason why relatively low fat diets are preferred in the modern treatment of diabetes mellitus.

Neutral fat and cholesterol esters increase in cells during fatty degeneration and infiltration. The widely held concept that *fatty degeneration* of liver, kidney, heart, muscles, and arteries was due entirely to the unmasking of the protoplasmic lipides (freeing them from adsorption compounds with proteins) is no longer tenable. Analyses of fatty degenerated tissues show that the total lipid is generally increased in these organs, although the lipid influx is smaller than that occurring in fatty infiltration.

Fatty infiltration of organs represents marked accumulation of lipid transported from other tissues. It is generally less serious and more easily reversed than fatty degeneration. Rapid infiltration of the liver causes a six to seven fold increase in total lipides, and great enlargement of the organ. The excess fat is transported from adipose tissues, as shown by the migration of foreign dietary fats and of deuterio fatty acids in experimental animals. Infiltration of the liver usually occurs when the glycogen stores of this organ have been depleted; the infiltration and the accompanying lipemia are sometimes relieved by high carbohydrate diets, or by measures which improve carbohydrate utilization. The relations of lipotropic factors to fatty infiltration have been discussed on page 219. The administration of lipotropic factors has been of little clinical value in the treatment of infiltrations. Fatty degeneration and infiltration of the liver occur in poisoning due to excess anterior pituitary extract, estrogens, phlorhizin, phosphorus, benzene, carbon tetrachloride, and chloroform; also during severe infectious processes, diphtheria, acute yellow atrophy of the liver,

pernicious anemia, diabetes, nephrosis, and the toxemias of pregnancy. Maximal protection of the liver against toxic agents is provided by a diet which has adequate caloric value and consists of 75 per cent carbohydrate, 20 per cent protein, and 5 per cent fat. In the nutritional muscular dystrophy of rabbits, there is a marked increase in muscle lipides, especially cholesterol and cholesterol esters.

The *fat necrosis* which occurs occasionally in abdominal tissues is caused by abnormal escape of pancreatic juice following rupture of the pancreas or its ducts. Steapsin digests fats in the damaged tissue areas, just as pancreatic enzymes lead to self-digestion of the pancreas after ischemia of that organ.

Demyelination and degeneration of nerves is accompanied by decrease in total lipides, breakdown of the phospholipides and cerebroside of the nerve sheaths, and cholesterol ester deposition. The lipides of the nerve sheaths are very sensitive to autolytic processes, to avitaminoses, and to such metabolic disorders as diabetes mellitus. Pronounced destruction of the myelin sheaths in the central nervous system occurs in the neurological disorder known as disseminated or multiple sclerosis. Hypoplasia of the adrenal cortex and accumulation of neutral fat and cholesterol esters accompany such brain anomalies as acrania, anencephalus, and hydrocephalus.

LIPIDOSES OR XANTHOMATOUS DISEASES

These diseases represent primary endogenous disturbances of lipid metabolism which involve the reticulo-endothelial system (the reticulo-endothelial cells of blood and lymph systems, the macrophages and the monocytes of connective tissues). During lipemias, these cells phagocytize lipid droplets or chylomicrons. Hand-Schüller-Christian syndrome and Gaucher's disease are related to disturbances of the reticulo-endothelial system.

Tumor-like accumulations of cholesterol esters, *xanthomas*, are produced by abnormal reticulo-endothelial cells. Hypercholesterolemia, at times, leads to the appearance of lipid-filled *foam cells* and *xanthoma cells* in reticulo-endothelial tissues. Xanthomatosis of this type is definitely secondary to lipemia and hypercholesterolemia; it occurs in certain cases of diabetes, xanthomatous biliary cirrhosis, and so forth. Eruptive xanthomatosis, or *xanthoma diabetorum*, is a symptom of marked lipemia. Hypercholesterolemia accompanies certain primary xanthomatoses of bile ducts, blood vessels, liver, lymph glands, skin, spleen, and tendons. Certain other xanthomas of the bones, brain, lungs, pituitary gland, and skin are associated with normal blood levels and are, therefore, attributed to local reticulo-endothelial disorders of cholesterol metabolism. When isolated in tissue cultures, the xanthoma cells from these tumors continue their abnormal accumulation of cholesterol esters. The *cholesteatoma*, or

large single cholesterol ester tumors of the central nervous system; the yellow deposits of cholesterol esters in eyelids, or *xanthelasmata*; and the deposits in cystic tumors of ectodermal origin, are not accompanied by hypercholesterolemia.

Insulin therapy aids the reabsorption of cholesterol deposits in diabetic conditions, while low-cholesterol and low-fat diets, lipocaic, lecithin, and thyroid have been used in the treatment of hypercholesterolemic xanthomatoses. Xanthomata of the bones and dura are said to respond to x-ray therapy better than those of the skin and tendons.

The *Hand-Schüller-Christian syndrome* is a familial disease, characterized by deposits of cholesterol and cholesterol esters in necrotized bone areas, particularly in the skull. In addition to osseous defects, these patients may have anemia, exophthalmos, hyperglycemia, and diabetes insipidus, suggestive of pituitary involvement. Many parts of the reticulo-endothelial system show abnormal sterol storage; low cholesterol diets occasionally improve the condition.

Niemann-Pick disease is a usually fatal congenital condition of childhood, especially prevalent among Hebrews. In this disease phospholipides, particularly sphingomyelins, are deposited in many tissues, including the bone marrow, brain, liver, and spleen; but the plasma phospholipide level is usually normal or subnormal. Anemia, emaciation, nystagmus, loss of vision, and enlargement of the liver and spleen result. The brain cerebroside appears to be abnormal in type, and cholesterol esters are deposited with them. In the closely related condition of *amaurotic idiocy* (Tay-Sachs disease), the phospholipide deposits tend to involve the brain more than the general viscera. Here, the brain cerebroside has an abnormally high carbohydrate content, since it contains increased quantities of ganglioside. The symptoms include idiocy, paralysis, and blindness. Both in Tay-Sachs and in Niemann-Pick disease, the macular region of the retina is white or gray with a central red spot.

Gaucher's disease is an inherited condition characterized by splenohepatomegaly, skeletal defects, anemia, leukopenia, and accumulation of cerebroside (so-called kerafin) in the liver, spleen, and other lymphatic and hematopoietic tissues which normally contain little of this lipid. The plasma contains only traces of cerebroside. In Gaucher's disease, the reticulo-endothelial tissues have acquired one of the characteristics of nerve tissue. The splenic cerebroside is abnormal in composition, since it contains glucose in place of galactose, but the cerebroside of the brain appears to be normal in composition. Diagnosis is assisted by x-ray findings, identification of the characteristic Gaucher cell in specimens of bone marrow, lymph nodes, or spleen, and isolation of cerebroside. The acute form of the disease is found in infants and children; advanced chronic cases may develop hemochromatosis (page 632).

In the fatal pediatric condition known as *sclerema neonatorum*, the subcutaneous fat becomes solidified and contains considerable free fatty acid.

CHOLEMIA AND CHOLURIA

Cholemia or increased concentration of bile salts in the blood occurs during obstructive jaundice, early hepatic disease, and bile peritonitis. When biliary obstruction occurs, bile salts appear in the lymph of the thoracic duct. In hepatitis, the liver assimilates bile salts from the blood with difficulty. Obstructive jaundice may be accompanied by blood bile salt levels as high as 30 mg. per cent, whereas the maximum level in hepatitis is near 10 mg. per cent. As hepatocellular disease progresses, the cholemia may diminish, owing to inhibition of bile acid synthesis in the liver. Cholemia causes lowered blood pressure and bradycardia.

Because of analytical difficulties in the determination of plasma bile salts, the urinary excretion of these substances is of greater clinical interest in studies of liver disease. Choluria appears during obstructive jaundice, and decreases with marked parenchymatous involvement. The urine of obstructive jaundice patients may contain as much as 100 mg. per cent of bile salts.

BIBLIOGRAPHY

CHEMISTRY

General

BULL, H. B. *Biochemistry of the Lipides*. New York, Wiley, 1937.

Fats

HILDITCH, T. P. *The Chemical Constitution of Natural Fats*. New York, Wiley, 1940.

JAMIESON, G. S. *Vegetable Fats and Oils*. Ed. 2. New York, Reinhold, 1943.

Phospholipides; Cerebrosides

ANDERSON, R. J. Structural peculiarities of acid-fast bacterial lipides. *Chem. Rev.*, 29 : 225, 1941.

GUGGENHEIM, M. *Die Biogenen Amine*. Ed. 3. New York, Nordemann, 1940.

THIERFELDER, H., and KLENK, E. *Chemie der Cerebroside und Phosphatide*. Berlin, J. Springer, 1930.

Sterides

American Association for the Advancement of Science. *The Chemistry and Physiology of Hormones*. Lancaster, Science Press, 1944.

ELDERFIELD, R. C. Chemistry of cardiac glycosides. *Chem. Rev.*, 17 : 187, 1935.

FIESER, L. F. *Chemistry of Natural Products Related to Phenanthrene*. Ed. 2. New York, Reinhold, 1937.

GILMAN, H., *et al.* *Organic Chemistry*. Ed. 2. Vol. 2. New York, Wiley, 1943.

- PIFFNER, J. J. The adrenal cortical hormones. *Adv. in Enzymol.*, 2 : 325, 1942.
- REED, C. I., *et al.* Vitamin D. Chicago, Univ. of Chicago Press, 1939.
- SOBOTKA, H. Chemistry of the Sterides. Baltimore, Wm. Wood, 1938.
- TSCHESCHE, R. Chemistry of plant heart poisons, toad toxins and saponins. *Ergeb. Physiol.*, 38 : 31, 1936.

Carotenoids; Tocopherols; Vitamin K

- DAM, H. Vitamin K, chemistry and physiology. *Adv. in Enzymol.*, 2 : 285, 1942.
- GILMAN, H., *et al.* Organic Chemistry. Vol. 2. New York, Wiley, 1938.
- MAYER, F. The Chemistry of Natural Coloring Matters. New York, Reinhold, 1943.
- REICHSTEIN, T., and SHOPPEE, C. W. The hormones of the adrenal cortex. *Vitamins and Hormones*, 1 : 345, 1943.
- SMITH, L. I. The chemistry of vitamin E. *Chem. Rev.*, 27 : 287, 1940.
- ZECHMEISTER, L. Stereochemistry of carotenoids and diphenylpolyenes. *Chem. Rev.*, 34 : 267, 1944.

METABOLISM

Fats

- BLOOR, W. R. Biochemistry of the Fatty Acids. New York, Reinhold, 1943.
- BURR, G. O., and BARNES, R. H. Non-caloric functions of dietary fats. *Physiol. Rev.*, 23 : 256, 1943.
- CARTER, H. E. Oxidation of branched-chain fatty acids. *Biol. Symposia*, 5 : 47, 1941.
- INGLE, D. J. Relationship of the adrenal cortex to the metabolism of fat. *J. Clin. Endocrinol.*, 3 : 603, 1943.
- MACKAY, E. M. The significance of ketosis. *J. Clin. Endocrinol.*, 3 : 101, 1943.
- McHENRY, E. W., and CORNETT, M. L. The role of vitamins in the anabolism of fats. *Vitamins and Hormones*, 2 : 1, 1944.
- McHENRY, E. W., and PATTERSON, J. M. Lipotropic factors. *Physiol. Rev.*, 24 : 128, 1944.
- SOSKIN, S., and LEVINE, R. Origin and regulation of ketone bodies. *Arch. Int. Med.*, 68 : 674, 1941; *Biol. Symposia*, 5 : 64, 1941.
- STADIE, W. C. The intermediary metabolism of fatty acids. *Physiol. Rev.*, 25 : 395, 1945.
- VERZAR, F., and McDougall, E. J. Absorption from the Intestine. New York, Longmans, Green, 1936.

Isotopes

- HAMILTON, J. G. The use of radioactive tracers in biology and medicine. *Radiology*, 39 : 541, 1942.
- KURBATOV, J. D., and POOL, M. L. Radioactive isotopes for the study of trace elements in living organisms. *Chem. Rev.*, 32 : 231, 1943.
- ROSS, J. F. Isotopes in medical investigation and therapy. *New England J. Med.*, 228 : 454, 482, 1943.

SCHOENHEIMER, R., and RITTENBERG, D. The study of intermediary metabolism with the aid of isotopes. *Physiol. Rev.*, 20 : 218, 1940.

Phospholipides

CHAIKOFF, I. L. Application of labeling agents to phospholipide metabolism. *Physiol. Rev.*, 22 : 291, 1942.

PAGE, I. H. Chemistry of the Brain. Springfield, Thomas, 1937.

SINCLAIR, R. G. Anabolism and function of the phospholipides. *Biol. Symposia*, 5 : 82, 1941.

Choline; Acetylcholine; Neuro-transmission

BEST, C. H., and LUCAS, C. C. Choline chemistry and significance as a dietary factor. *Vitamins and Hormones*, 1 : 1, 1943.

BROWN, G. L. Transmission at nerve endings by acetylcholine. *Physiol. Rev.*, 17 : 485, 1937.

ECCLES, J. C. Synaptic and neuromuscular transmission. *Physiol. Rev.*, 17 : 538, 1937.

FULTON, J. F., and NACHMANSOHN, D. Acetylcholine and the physiology of the nervous system. *Science*, 97 : 569, 1943.

GLICK, D. Nature and significance of choline esterase. *Biol. Symposia*, 5 : 213, 1941.

GRIFFITH, W. H. Nutritional importance of choline. *J. Nutrition*, 22 : 239-53, 1941.

Sterides

COOK, R. P. Cholesterol metabolism. *Nutrition Abstr. & Rev.*, 12 : 1, 1942.

JOSEPHSON, B. Circulation of the bile acids. *Physiol. Rev.*, 21 : 463, 1941.

MARRIAN, G. F. Intermediary metabolism of the steroid hormones. *Harvey Lect.*, 34 : 37, 1938-39.

PINCUS, G., and PEARLMAN, W. H. The intermediate metabolism of the sex hormones. *Vitamins and Hormones*, 1 : 294, 1943.

SOBOTKA, H. Physiological Chemistry of the Bile. Baltimore, Wm. Wood, 1937.

WEINHOUSE, S. The blood cholesterol. *Arch. Path.*, 35 : 438, 1943.

Action of Light; Chemistry of the Eye

BELLOWS, J. G. Cataract and Anomalies of the Lens. St. Louis, Mosby, 1944.

BLUM, H. F. Photodynamic Action and Diseases Caused by Light. New York, Reinhold, 1940.

DUGGAR, B. M. Biological Effects of Radiation. New York, McGraw-Hill, 1936, (2 vol.).

ELLIS, C., and WELLS, A. A. The Chemical Action of Ultraviolet Rays. Ed. 2. New York, Reinhold, 1941.

KRAUSE, A. C. The Biochemistry of the Eye. Baltimore, Johns Hopkins Press, 1934.

(Series of authors.) Visual Mechanisms. *Biol. Symposia*, Vol. VII. Lancaster, Cattell Press, 1942.

WALD, G. The photoreceptor function of the carotenoids and vitamins A. *Vitamins and Hormones*, 1 : 245, 1943.

PATHOLOGY

Lipidoses; Lipide Degeneration

- COWDRY, E. V. Arteriosclerosis. New York, Macmillan, 1933.
- HIRSCH, E. F., and WEINHOUSE, S. The role of lipides in atherosclerosis. *Physiol. Rev.*, 23 : 185, 1943.
- HUEPER, W. C. Arteriosclerosis. *Arch. Path.*, 38 : 162, 245, 350, 1944.
- (Series of authors.) Ageing and Degenerative Diseases. *Biol. Symposia*, Vol. XI. Lancaster, Cattell Press, 1945.
- THANNHAUSER, S. J. Lipidoses. London, Oxford Univ. Press, 1940.

Cholinergic Diseases

- GAMMON, G. D., *et al.* Nature of certain diseases of the voluntary muscles, *Biol. Symposia*, 3 : 291, 1941.

Carcinogenesis

- BURK, D., and WINZLER, R. J. Vitamins and cancer. *Vitamins and Hormones*, 2 : 305, 1944.
- DODDS, E. C. Hormones in cancer. *Vitamins and Hormones*, 2 : 353, 1944.
- RUSCH, H. P. Extrinsic factors that influence carcinogenesis. *Physiol. Rev.*, 24 : 177, 1944.
- STERN, K. Biochemistry of Malignant Tumors. New York, Chemical Pub. Co., 1943.

Miscellaneous

- CONN, J. W. Obesity : etiological aspects. *Physiol. Rev.*, 24 : 31, 1944.
- HORRALL, O. N. Bile, Its Toxicity, and Relation to Disease. Chicago, Univ. of Chicago Press, 1938.
- IVY, A. C. Applied physiology of bile secretion and bile salt therapy. *J. A. M. A.*, 117 : 1151, 1941.
- NEWBURGH, L. H. Obesity : energy metabolism. *Physiol. Rev.*, 24 : 1, 1944.
- RONY, H. R. Obesity and Leanness. Philadelphia, Lea and Febiger, 1940.
- STADIE, W. C. Fat metabolism in diabetes mellitus. *Ann. Int. Med.*, 15 : 783, 1941.
- WELLS, H. G., and LONG, E. R. The Chemistry of Tuberculosis. Ed. 2. Baltimore, Williams and Wilkins, 1932.

CHAPTER V

CARBOHYDRATES



CHEMISTRY

"It is necessary for science to frequently examine established opinions and reopen issues supposed to have been closed." — MORRIS R. COHEN

CLASSIFICATION

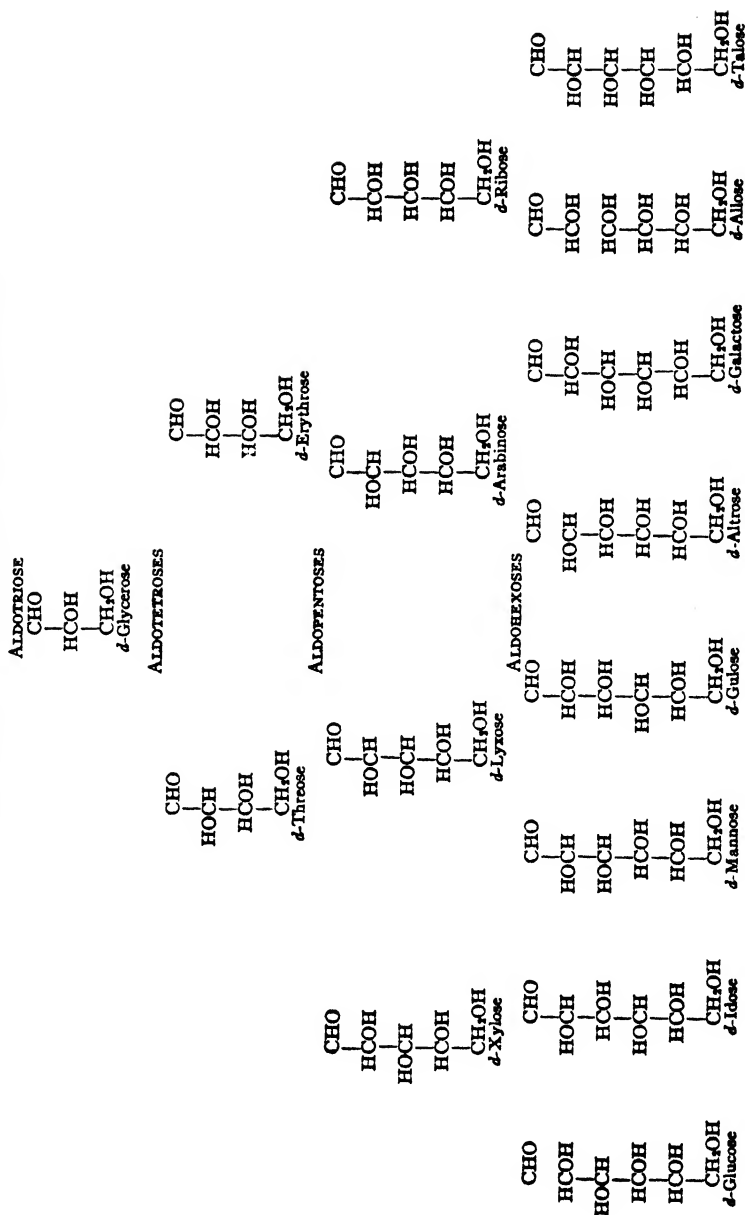
This important group of substances includes aliphatic hydroxy-carbonyl compounds and their derivatives. In addition to the classification given in Table 45, the aldose and ketose *monosaccharides* are divided into dioses, trioses, tetroses, pentoses, hexoses, and heptoses according to the number of carbon atoms. The term carbohydrate is suggested by the empirical formula of the most common monosaccharides, $(\text{CHOH})_n$; amino, desoxy, and anhydro sugars do not conform to this formula. Aldoses, ketoses, amino sugars, desoxy sugars, and uronic acids reduce ordinary alkaline copper reagents. Certain anhydro sugars, oligosaccharides, and sugar esters also reduce alkaline oxidizing reagents, while carboxylic acids, sugar alcohols, and polysaccharides are non-reducing. A reducing sugar has a potential aldehyde or ketone radical; all sugars with more than three carbon atoms are actually heterocyclic compounds whose rings contain from two to six carbon atoms and an oxygen bridge at the potential carbonyl radical.

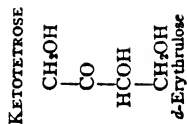
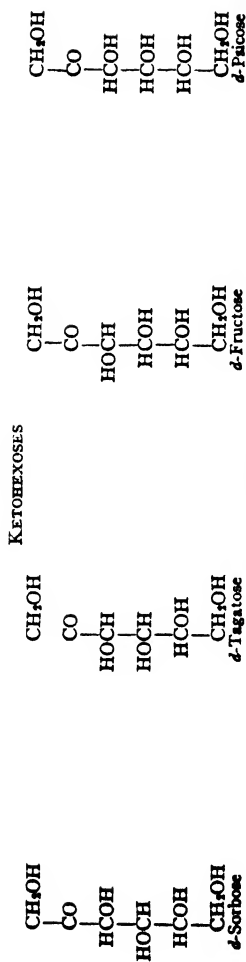
Oligosaccharides are compounds formed from n monosaccharide units by the removal of $n - 1$ molecules of water; *polysaccharides* are formed similarly by the removal of n molecules of water from n monosaccharide units. In the case of oligosaccharides n is any integer from 2 to 6, and in the case of polysaccharides it is greater than 6 (Table 57, page 291). Such complex carbohydrates are hydrolyzed to monosaccharides by acids and by enzymes.

STEREISOMERISM

Carbohydrates contain asymmetric carbon atoms (carbon atoms whose valences are satisfied by four different radicals); hence, they exist in stereoisomeric forms, many of which are optically active. For compounds having n asymmetric carbon atoms, the number of stereoisomers is 2^n . *d*-Forms

TABLE 44
STEREOISOMERISM OF *d*-ALDOSES AND *d*-KETOSES¹





¹ The aldodiose, glycolic aldehyde (CHO—CH₂OH), and the ketotriose, dihydroxyacetone (CH₂OH—CO—CH₂OH), are not optically active.

TABLE 45

CLASSIFICATION OF CARBOHYDRATES

1. *Monosaccharides**Aldoses*, carbohydrates with potential aldehyde radicals*Ketoses*, carbohydrates with potential ketone radicals*Amino sugars*, in which NH_2 replaces an —OH radical*Desoxy sugars*, in which H replaces an —OH radical*Anhydro sugars*, sugar anhydrides with two ring systems*Derivatives of monosaccharides**Sugar acids*

Monocarboxylic acids

Dicarboxylic acids

Uronic acids, with both carboxyl and carbonyl radicals

Alduronic acids (aldose acids)

Keturonic acids (ketose acids)

*Sugar esters**Sugar ethers**Glycosides*, with ether linkage at the reducing carbon2. *Oligosaccharides*, or compound carbohydrates containing two to six monosaccharide units*Disaccharides*, with two monosaccharide units*Trisaccharides*, with three monosaccharide units3. *Polysaccharides*, or compound carbohydrates of high molecular weight*Simple or homogeneous polysaccharides*

Pentosans, polymers of anhydropentoses

Hexosans, polymers of anhydrohexoses

Complex or mixed polysaccharides, containing several types of anhydro sugar units

Polyuronides, with one unit an anhydro uronic acid

Hemicellulose polyuronides

Polysaccharide acids, with free carboxyl radicals

Pectins

Gums (or mucilages)

Prosthetic polysaccharides of animal and bacterial proteins

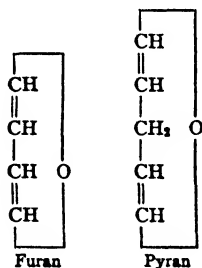
of aldose and ketose stereoisomers are given in Table 44; for each of these there is also a corresponding *l*-form or mirror image. To write the formula for an *l*-sugar, it is necessary to transpose the radicals on each asymmetric carbon atom of the formula for the *d*-sugar. All formulae for *d*-sugars have the —OH radical of the second last carbon atom (counting from the reducing radical) projected to the right. The *d*- and *l*-forms of sugars account for four ketopentoses, eight ketohexoses, eight aldopentoses, and sixteen aldohexoses. Monosaccharides of considerable biochemical interest include *d*-glucose, *d*-fructose, *d*-galactose, *d*-mannose, *d*-xylose, *d*-ribose, and *l*-arabinose.

The arrangement of monosaccharides given in Table 44 is a *cis-trans* classification, which correlates certain chemical properties of sugars. Sugars whose substituents on the three carbons adjacent to the reducing carbon are completely *trans* are at the extreme left of the table, and completely *cis* sugars at the extreme right. Organic chemistry texts usually present the Wohl-Freudenberg arrangement, which is based on synthetic interrelations.

TABLE 46
EQUILIBRIUM FORMS OF AN ALDOHEXOSE

Lactols					
I	II	III	IV	V	VI
$\begin{array}{c} \text{CHO} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CH}_2\text{OH} \\ \text{Aldehyde} \end{array}$	$\begin{array}{c} \text{CHOH} \\ \\ \text{COH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CH}_2\text{OH} \\ \text{Enol} \end{array}$	$\begin{array}{c} \text{CHOH} \\ \\ \text{HC} \text{---} \text{O} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CH}_2\text{OH} \\ \text{Ethylene Oxide} \end{array}$	$\begin{array}{c} \text{CHOH} \\ \\ \text{CHOH} \text{---} \text{O} \\ \\ \text{CHOH} \\ \\ \text{HC} \text{---} \text{O} \\ \\ \text{CHOH} \\ \\ \text{CH}_2\text{OH} \\ \text{Furanose}^1 \end{array}$	$\begin{array}{c} \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \text{---} \text{O} \\ \\ \text{CHOH} \\ \\ \text{HC} \text{---} \text{O} \\ \\ \text{CH}_2\text{OH} \\ \text{Pyranose}^1 \end{array}$	$\begin{array}{c} \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \text{---} \text{O} \\ \\ \text{CHOH} \\ \\ \text{CHOH} \\ \\ \text{H}_2\text{C} \\ \text{Septanose} \end{array}$

¹ The terms furanose and pyranose are derived from the following reference heterocycles:



Glycerose and dihydroxyacetone are the only carbohydrates which have the simple aliphatic structures pictured in the table; the others exist as heterocyclic compounds (types III to VI, in Table 46). These cyclic forms are internal hemiacetals or *lactols*; the rings close through attachment of oxygen at the potential aldehyde or ketone radical. Aqueous solutions of a given sugar contain several lactols in equilibrated mixture. Trioses are correctly represented as aldehyde forms (I), while the enolic forms (II) are unstable intermediates of sugars in alkaline solution. Ethylene oxide and septanose lactols have been detected in certain sugar

solutions, but the most common cyclic forms of ordinary sugars are pyranose (V) and furanose (IV). Methylation studies have demonstrated a pyranose structure for ordinary pentoses and hexoses; the furanoses are more labile forms of these sugars. Thus, ordinary *d*-glucose is *d*-glucopyranose, and the reactive form of *d*-fructose found in cane sugar is *d*-fructofuranose. Acidity favors the production of furanoid forms in sugar solutions; alkalinity leads to rapid equilibration of lactols and rupture of their rings.

α - β ISOMERISM

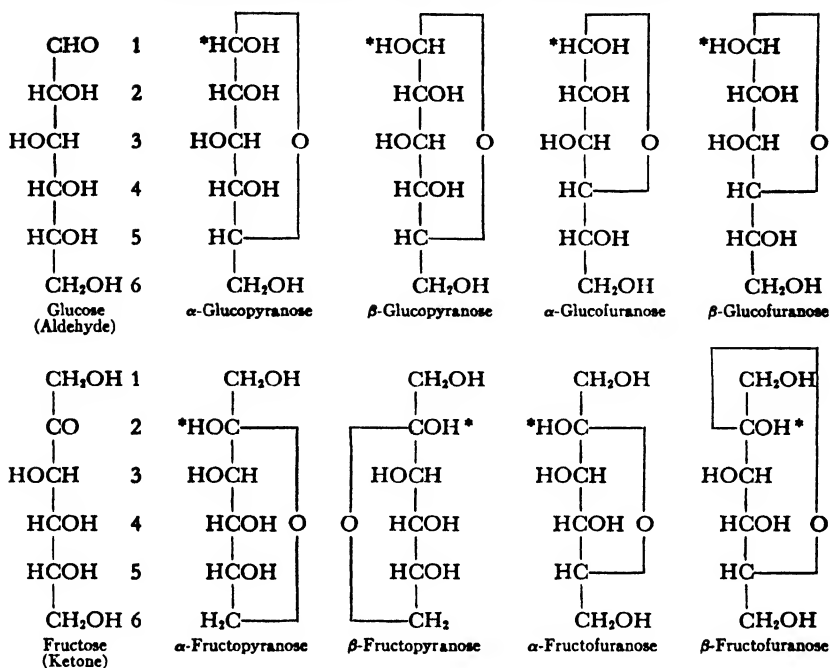
Ring closure causes the reducing carbon atom to become asymmetric. Each lactol, therefore, exists in two forms called α - and β -isomers, which double the number of stereoisomers cited on page 264. The α - and β -isomers differ in respect to the position of the —OH radical at the reducing carbon. These relations and the system of numbering sugar carbons are illustrated in Table 47. In the table, the asymmetric reducing carbon atom is indicated by an asterisk. α -*d*-Glucopyranose, ordinary crystalline *d*-glucose, has the hydroxyl radicals of carbons 1 and 2 in *cis* position, as indicated by its reaction with boric acid to form a highly dissociated glucoboric acid. This reaction is a property of *cis* hydroxyls.

OPTICAL ACTIVITY

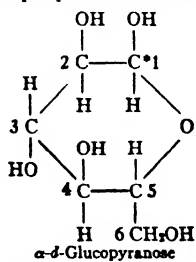
When a beam of light is passed through a prism of calcite or of Iceland spar it becomes plane polarized; that is, its vibration is limited to one plane. Solutions of optically active substances rotate the plane of polarized light either to the right or to the left. The angle of rotation is measured in the polariscope; in this instrument, the emerging beam of light passes through a second rotatable prism of calcite. Optically active substances are classified as dextrorotatory (*d*-), or levorotatory (*l*-), according to the direction in which they rotate polarized light. Isomers which are mirror images of each other are called *enantiomorphs*, *optical isomers*, or *d*- and *l*-forms; they rotate polarized light equally, but in opposite directions. The upper halves of the formulae for *meso isomers* of sugar acids and alcohols are mirror images of the lower halves. Because of intramolecular compensation, they do not rotate polarized light. Racemic mixtures contain equal quantities of optical isomers, and are optically inactive.

In sugar nomenclature, the symbols *d*- and *l*- do not conform to the general chemical usage outlined above; instead, they designate the structural relations of the sugar to the *d*- and *l*-forms of glycerose (Table 44). On this basis, the naturally occurring amino acids all belong to the *l*-series regardless of the direction of rotation. While the *d*- and *l*-forms of sugars are optical isomers, their symbols do not indicate the direction of the rotation of polarized light. The enantiomorphs of a given sugar react differently with enzymes and with asymmetric chemical reagents *in vitro*.

TABLE 47
FORMS OF *d*-GLUCOSE AND *d*-FRUCTOSE ¹



¹ Haworth has introduced improved perspective formulae for sugar lactols, for example:



Perspective formulae have not been employed in this text because the vertical formulae are more easily visualized by students.

The *specific rotation* (Table 48) is the angle through which 1 decimeter of a 100 per cent solution of a substance rotates the plane of polarized monochromatic light (usually the D line of sodium). In practice, the optical rotation is measured in dilute solution and the specific rotation is calculated by use of the formula:

$$[\alpha]_D^{20} = \frac{\alpha \times 100}{l \times c}$$

TABLE 48

A. ANALYTICAL PROPERTIES OF SUGARS

	M. P. OF PHENYL- OSAZONE (°C)	SPECIFIC ROTATION [α] _D ²⁰			REDUCING EQUIVALENTS ¹ (<i>d</i> -Glucose = 1.00)		
		α - Form	Equilib- rium	β - Form	Folin-Wu Method	Sumner Method	Sumner Folin-Wu
<i>d</i> -Arabinose	163		- 105		0.69	1.13	1.65
<i>l</i> -Arabinose	166	+ 75.5	+ 105	+ 190.5	0.87	1.14	1.3
<i>l</i> -Ascorbic acid			+ 24		0.55 ²	0.44	0.8
Cellobiose	200		+ 35		0.53	0.83	1.75
<i>d</i> -Chitose	202		+ 32				
2-Desoxy- <i>d</i> -ribose			- 50				
Dextrin ³			+ 195				
Digitoxose			+ 46.5				0.85
*Dihydroxyacetone	132				0.57	0.66	1.15
* <i>d</i> -Fructose	208	- 21	- 92	- 133.5	0.91	0.99	1.1
* <i>d</i> -Fructose-6-phosphoric acid ⁴			+ 24, + 36				
<i>d</i> -Fructose-1,6-diphosphoric acid ⁵			+ 3.5				
<i>d</i> -Fructo-1-uronic acid			- 75.5		0.79	0.87	1.1
<i>d</i> -Fructo-6-uronic acid						0.98	
<i>l</i> -Fructo-6-uronic acid						0.95	
<i>l</i> -Fucose	178		- 75.5		0.45	1.00	2.2
* <i>d</i> -Galactose	201	+ 150.5	+ 80	+ 53	0.78	0.97	1.25
<i>l</i> -Galactose	195		- 78				
<i>d</i> -Galacturonic acid · H ₂ O	131	+ 98	+ 51	+ 24.5	0.65	0.87	1.35
Gentiobiose	180	+ 31	+ 9.5	- 11	0.54	0.66	1.2
<i>d</i> - α -Glucoheptose	195	+ 45	- 20.5	- 29	0.62	0.87	1.45
<i>d</i> -Glucosamine hydrochloride		+ 100	+ 72.5	+ 25	0.79	0.33	0.4
* α - <i>d</i> -Glucosan	208		+ 70		0.19 ²	0.22	1.15
β - <i>d</i> -Glucosan			- 66				
* <i>d</i> -Glucose	208	+ 112	+ 52.5	+ 19	1.00	1.00	1.00
<i>d</i> -Glucose-1-phosphoric acid ⁶			+ 120				
* <i>d</i> -Glucose-6-phosphoric acid ⁷	140		+ 27				
* <i>d</i> -Glycerose	132		+ 14				
Glycogen			+ 196				
<i>d</i> -Glycuronic acid	202	+ 82	+ 36.5	- 5	0.71 ²	0.91	1.3
Inulin			- 40				
*Lactose · H ₂ O	200	+ 83.5	+ 52.5	+ 35	0.47	0.77	1.65
<i>d</i> -Lyxose	163	+ 5.5	- 14	- 72.5	0.96	1.16	1.2
*Maltose · H ₂ O	206	+ 133	+ 130	+ 112.5	0.42	0.76	1.8
<i>d</i> - α -Mannoheptose	200	+ 120	+ 64.5	+ 42.5	0.61	0.83	1.35
<i>d</i> -Mannoketoheptose	200		+ 29.5		0.69	1.00	1.45
* <i>d</i> -Mannose	208	+ 29.5	+ 14	- 17	0.45 ²	0.97	2.15
<i>d</i> -Mannuronic acid			- 24	- 48			
Melezitose			+ 89				
Melibiose · 2 H ₂ O	178		+ 126.5	+ 110.5	0.49	0.63	1.3
α -Methyl glucoside			+ 159				
β -Methyl glucoside			- 34				
*Raffinose · 5 H ₂ O			+ 104.5				
<i>l</i> -Rhamnose · H ₂ O	182	- 8.5	+ 8	+ 54	0.32 ²	0.98	3.05
<i>d</i> -Ribose	163		- 19.5		0.62	1.08	1.75
Sedoheptose	197		- 146				
<i>d</i> -Sorbse	168		+ 43		0.78	0.99	1.25
<i>l</i> -Sorbse	164		- 43		0.83 ²	0.95	1.15
<i>l</i> -Sorbo-1-uronic acid			- 48				
<i>l</i> -Sorbo-6-uronic acid			- 13.5		0.70	0.80	1.15
*Sucrose			+ 66.5				
<i>d</i> -Tagato-1-uronic acid			- 6				
<i>l</i> -Tagato-1-uronic acid					0.61	0.68	1.1
<i>d</i> -Tagato-6-uronic acid					0.76	0.96	1.25
<i>l</i> -Tagato-6-uronic acid						0.85	
Trehalose · 2 H ₂ O			+ 178.5				
<i>l</i> -Xyloketose	163		+ 33				
<i>d</i> -Xylose	163	+ 93.5	+ 19	- 20	0.98	1.14	1.15
<i>l</i> -Xylose			- 19		0.98	1.18	1.2

¹ These are reducing equivalents for equal weights of sugars (0.1 to 0.2 mg. per ml. for the Folin-Wu method, and 0.5 mg. per ml. for Sumner's method). See page 281 for explanation.

² The equivalents of these sugars in the Folin-Wu method vary with the concentration.

³ Purified commercial product. ⁴ Neuberg ester. ⁵ Harden-Young ester. ⁶ Cori ester. ⁷ Robinson ester.

⁸ Readily fermentable by fresh baker's yeast.

⁹ Fermented slowly, or only occasionally by fresh baker's yeast.

TABLE 48 (Cont.)
B. REDUCING EQUIVALENTS FOR OTHER
ANALYTICAL METHODS

	BERTRAND ^a METHOD	SHAFFER-HARTMAN METHOD	HAGEDORN- JENSEN METHOD
<i>d</i> -Arabinose		0.73	
<i>l</i> -Arabinose	1.04	0.83	0.94
Cellobiose	0.70		
<i>d</i> -Fructose	0.98	0.90-0.96	0.98
<i>d</i> -Fructose-6-phosphoric acid	0.66		
<i>l</i> -Fucose		0.63	
<i>d</i> -Galactose	0.95	0.80-0.84	0.79
<i>d</i> -Glucose	1.00	1.00	1.00
Lactose · H ₂ O	0.70	0.48-0.53	0.66
Maltose · H ₂ O	0.55	0.42	0.75
<i>d</i> -Mannose	1.00	0.75-0.81	1.00
<i>l</i> -Rhamnose		0.83	
<i>l</i> -Sorbose	0.75		
<i>d</i> -Xylose	0.99	0.97	0.92

^a Equivalents for 10 mg. portions of sugars.

where α is the observed rotation, l is the length of the polariscope tube (in decimeters), and c is the number of grams of substance per 100 ml. of the solution used. The *molecular rotation* of a sugar is the product of its specific rotation and its molecular weight.

Certain salts alter the optical rotation of carbohydrate solutions. Marked rotational changes are produced by tungstates, molybdates, and borates; these anions readily form coordination compounds with sugars. The effect has been utilized to verify the asymmetry of certain carbohydrates which have small specific rotations.

MUTAROTATION

The optical rotation of a freshly dissolved sugar often changes gradually until an equilibrium is attained. The equilibrium may be established rapidly by the addition of a small amount of alkali to the sugar solution. Mutarotation is most rapid in a medium in which cations and anions are available for combination with the sugar; protons (H⁺) are transferred from hydroxyl radicals to the cyclic oxygen to produce isomers.

An important cause of mutarotation is the interconversion of α - and β -forms of lactols. This reaction is a reversible rearrangement at the reducing carbon, and the equilibrium is characteristic for each sugar (Table 48). In equilibrated aqueous aldose solutions, that form of the α - β mixture preponderates which has the hydroxyl radicals of carbons 1 and 2 in *trans* arrangement, as in β -*d*-glucopyranose (Table 47). A number of the important properties of sugars depend on *cis-trans* relations. It is regrettable that the α - β nomenclature does not provide an indication of these structural relations at carbons 1 and 2; it has been the

practice of sugar chemists to designate the most dextrorotatory forms of *d*-lactols and the most levorotatory forms of *l*-lactols as α -isomers. The student will find that α and β designations of sugar derivatives do not always conform to chemical reactivities or to enzyme specificity.

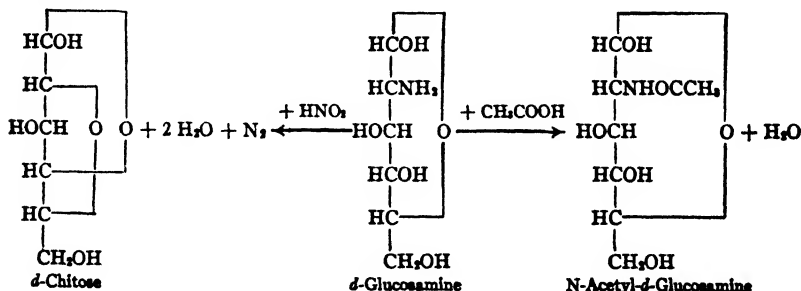
Another factor in mutarotation is rearrangement at the carbon atom adjacent to the reducing carbon of sugars, or to the carboxyl radical of sugar acids. This process, termed *epimerization*, is rapid in alkaline (pyridine) solutions.

A third factor in mutarotation is the interconversion of various lactols (furanose-pyranose interchange). It is not possible to transform *d*-sugars to *l*-sugars by such simple means. Mutarotation in solutions of sugar acids is due to a similar formation and interconversion of lactones.

MONOSACCHARIDES

The distribution and the nomenclature of common monosaccharides are summarized in Table 49. Analytical properties of several sugars are given in Table 48; the sugars fermented by fresh baker's yeast are indicated by an asterisk. Pentoses are not fermented, although they are easily metabolized by many bacteria. The fermentable sugars are closely related to *d*-glucose. *d*-Mannose is an epimer of *d*-glucose, and has the hydroxyls at carbons 2 and 3 in *cis* position; sugars which have this arrangement show a number of peculiar chemical and physical properties.

The 2-amino sugars reduce alkaline copper reagents, but they react feebly with nitrophenolic reagents and give none of the usual color tests for monosaccharides (page 277). Nitrous acid converts amino sugars to anhydro sugars and nitrogen; acylation with acetic acid produces the N-acetyl amino sugars found in nature.



The *desoxy sugars* are monosaccharides which have one or more hydroxyl radicals replaced by hydrogen. Those which have $-\text{CH}_3$ in place of a terminal $-\text{CH}_2\text{OH}$ radical are termed methylpentoses, methylhexoses, and so forth, a terminology easily confused with that of the methyl ethers of sugars whose linkage is not $-\text{C}-\text{CH}_3$, but rather $-\text{O}-\text{CH}_3$.

TABLE 49
MONOSACCHARIDES OF BIOCHEMICAL IMPORTANCE

SUGAR	STRUCTURE OF CRYSTALLINE SUGAR	DISTRIBUTION IN NATURE
Trioses		
<i>D</i> -Glycerose (glyceraldehyde)	Aldehyde form	Intermediate metabolite of animals and plants (as phosphoric ester).
Dihydroxyacetone	Ketone form	Intermediate metabolite of animals and plants (as phosphoric ester).
Pentoses		
<i>D</i> -Xylose	α - <i>D</i> -Xylopyranose	In the plant polysaccharide, xylan.
<i>D</i> -Arabinose	β - <i>D</i> -Arabopyranose	In polysaccharides of tubercle bacilli.
<i>L</i> -Arabinose	β - <i>L</i> -Arabopyranose	In polymeric arabans of seeds and fruits; also in plant gums and pectins.
<i>D</i> -Ribose	Ribopyranose	In plants and animals (in furanoid form) as ribonucleic acids, ribonucleotides.
<i>L</i> -Xyloketose		In urine of pentosuric patients.
Hexoses		
<i>D</i> -Glucose (dextrose)	α - <i>D</i> -Glucopyranose (<i>cis</i> form)	Most important free monosaccharide of animals and plants; in animals as phosphate esters, disaccharides, and glycogen; in plants as oligosaccharides, phosphate esters, glycosides, cellulose, and starch.
<i>D</i> -Mannose	β - <i>D</i> -Mannopyranose (<i>cis</i> form)	In animals as prosthetic polysaccharides of albumins, globulins, mucoids; in the prosthetic polysaccharide of tubercular protein; in plants as polymeric mannosans and gums.
<i>D</i> -Galactose	α - <i>D</i> -Galactopyranose (<i>cis</i> form)	In animals as prosthetic polysaccharides of proteins, as lactose, and as cerebroside; in prosthetic polysaccharide of tubercle bacilli; in plants as oligosaccharides, glycosides, polymeric galactans, pectins, gums, mucilages, and agar.
<i>L</i> -Galactose		In plant mucilages.
<i>D</i> -Fructose (levulose)	β - <i>D</i> -Fructopyranose (<i>trans</i> form)	The sweetest sugar; in animals (in furanoid form) as phosphate esters; in plants, honey, and fruit juices as free fructose; and (in furanoid form) as a component of cane sugar, oligosaccharides, and fructosans (inulin).
Heptoses		
<i>D</i> -Mannoketoheptose (<i>D</i> -mannoheptulose)		In avocados as the free sugar.
Sedoheptose		In stonecrop as the free sugar.
Amino sugars		
Glucosamine (2-aminoglucose) (chitosamine)	Glucosaminopyranose	As <i>N</i> -acetyl- <i>D</i> -glucosamine in prosthetic polysaccharides of animal albumins, globulins, sulfomucins, and mucoids; also as polymeric chitin or skeletal substance of insects, crustacea and fungi.

TABLE 49 (Cont.)

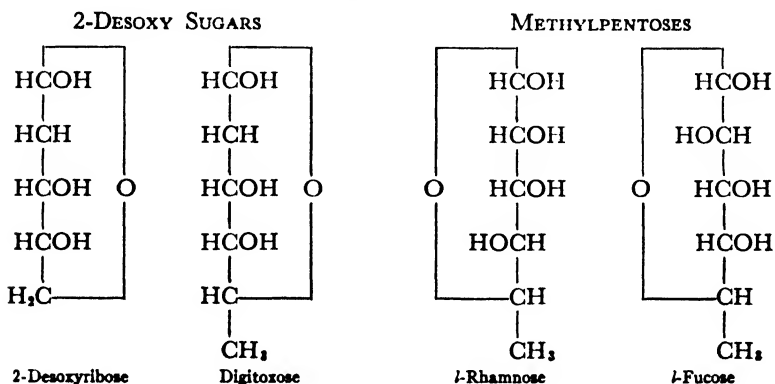
MONOSACCHARIDES OF BIOCHEMICAL IMPORTANCE

SUGAR	STRUCTURE OF CRYSTALLINE SUGAR	DISTRIBUTION IN NATURE
<i>Amino sugars</i> (Cont.)		
Galactosamine (2-aminogalactose) (chondrosamine) .	Galactosaminopyranose	As N-acetyl-d-galactosamine in prosthetic polysaccharides of animal chondroproteins.
<i>Desoxy sugars</i>		
Desoxyribose (2-desoxy-d-ribose)	Desoxyribopyranose	In desoxyribonucleic acids.
Digitoxose	Digitoxopyranose	As digitalis glycosides in plants.
l-Rhamnose (6-desoxy-l-mannose)	α -l-Rhamnopyranose (trans form)	In plants as flavon and saponin glycosides, also in gums and mucilages.
l-Fucose (6-desoxy-l-galactose)	α -l-Fucopyranose	In polymeric fucosans of marine algae.
<i>Sugar alcohols</i>		
Glycerol		In animals and plants as lipides and phosphate esters.
Erythritol		In lichens, algae, and fungi.
d-Arabitol		In lichens, algae, and fungi.
d-Ribitol		In flavins.
d-Mannitol		In leaves, fruits, roots and sap of plants; also in algae, fungi, lichens, and bacteria.
d-Sorbitol		In fruits, leaves and roots of certain plants, and in fungi.
Dulcitol		Widely distributed in plants and algae.
<i>Sugar acids</i>		
d-Gluconic acid		Phosphogluconic acid is an intermediary metabolite of animals and plants.
d-Glycuronic acid	β -d-Glycuronopyranose (trans form)	In animals as prosthetic polysaccharides of sulfomucins, mucoids, and chondroproteins; also excreted as glucuronides (glycuronates) of drugs; in plants as gums and hemicelluloses; as type specific polysaccharides of bacteria.
d-Mannuronic acid	β -d-Mannuronopyranose (cis form)	In polysaccharides of algae.
d-Galacturonic acid	α -d-Galacturonopyranose (cis form)	In polymeric pectins, gums, mucilages, and certain hemicelluloses.
l-Ascorbic acid	2,3-Enediol of keto-l-gulono- γ -lactone	In plants as free acid.
d-Fructo-1-uronic acid (2-keto-gluconic acid) . . .		Intermediate metabolite of acetic bacteria.
l-Sorbo-6-uronic acid (5-keto-gluconic acid)		Intermediate metabolite of acetic bacteria.

(page 286). Formulae of important naturally occurring desoxy sugars are given in Table 50. The 2-desoxy sugars are more reactive and less

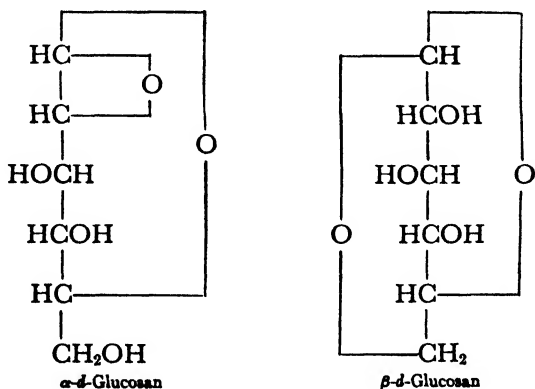
stable than ordinary aldoses; in the free state, they change to green tars. They react as aldehydes rather than as lactols, and they do not form osazones. Desoxyribose is responsible for the Feulgen nuclear reaction (page 515).

TABLE 50
DESOXY SUGARS



Anhydro Sugars

The anhydro sugars have two ring systems: a lactol ring, and a second oxygen bridge produced by the elimination of water from two *cis* hydroxyl radicals. The better known anhydro derivatives of *d*-glucose serve as illustrations:



α -d-Glucosan is produced by heating α -d-glucose *in vacuo*; β -d-glucosan, also called levoglucosan, is formed similarly from β -d-glucose or by the dry distillation of starch or cellulose under reduced pressure.

The properties of these sugars depend on the stability of the second oxygen bridge. α -*D*-Glucosan, in aqueous solution, is readily hydrolyzed to glucose, while levoglucosan is a stable non-reducing sugar. Chitose, the reducing anhydro sugar related to glucosamine (page 270), is very resistant to hydrolysis. α -*D*-Glucosan, and the anhydro sugars related to it, tend to polymerize to non-reducing carbohydrates of high molecular weight; the latter are similar to the polysaccharides, which also have empirical formulae of anhydro sugar polymers (Table 57, page 291).

REACTIONS OF REDUCING SUGARS IN ALKALINE SOLUTION

Typical effects of alkalis appear in sugar solutions free of oxidizing agents. The first reaction in alkaline solution appears to be the formation of *coordination compounds* with inorganic hydroxides or carbonates; such compounds of sugars and the alkaline earth or heavy metal hydroxides are rather insoluble. At a sufficiently high pH, cations may also react with sugars to form salts inasmuch as carbohydrates are feebly ionized acids (Table 1, page 4).

These effects of alkalis accelerate *interconversion* of the lactol and enolic forms of reducing sugars (Table 46, page 265). In the presence of suitable inorganic substances which form coordination compounds, similar interconversions take place slowly below pH 7.0. *D*-Glucose, *D*-mannose, and *D*-fructose are interconvertible in dilute alkali at room temperature. The phenomenon, known as the Lobry de Bruyn and van Eckenstein rearrangement, applies generally to reducing sugars. The reaction is reversible; true equilibrium is not attained, because of secondary irreversible reactions. The interconversion has been postulated as occurring via enolic forms of stereoisomerically related sugars. The original lactol produces the enolic form, and subsequently isomeric lactols are formed. Enolization begins at the reducing carbon and extends to adjacent carbon atoms. These complicated reactions are illustrated in Figure 5.

At higher temperatures, alkaline solutions cause *degradation* or *fission* of the carbon chains of sugar molecules, which leads to secondary decomposition and acid formation. Sterilization of *D*-glucose solutions at 115° C., for twenty minutes, destroys about 28 per cent of the sugar. Concentrated alkali produces drastic degradation of sugars, with the formation of triose derivatives (glycerose, dihydroxyacetone, and methyl glyoxal). In alkaline solutions, lactic and saccharinic acids are formed by a process of *dismutation* (simultaneous oxidation and reduction) of two molecules or fragments of the sugar. Saccharinic acids are, therefore, desoxy sugar acids in which one —CHOH radical has been converted to carboxyl and a second one to —CH₃ or —CH₂. Some saccharinic acids have branched chains. The lactic acid of muscles is the dextrorotatory *l*-form, whereas bacterial fermentation usually produces the racemic and *d*-forms.

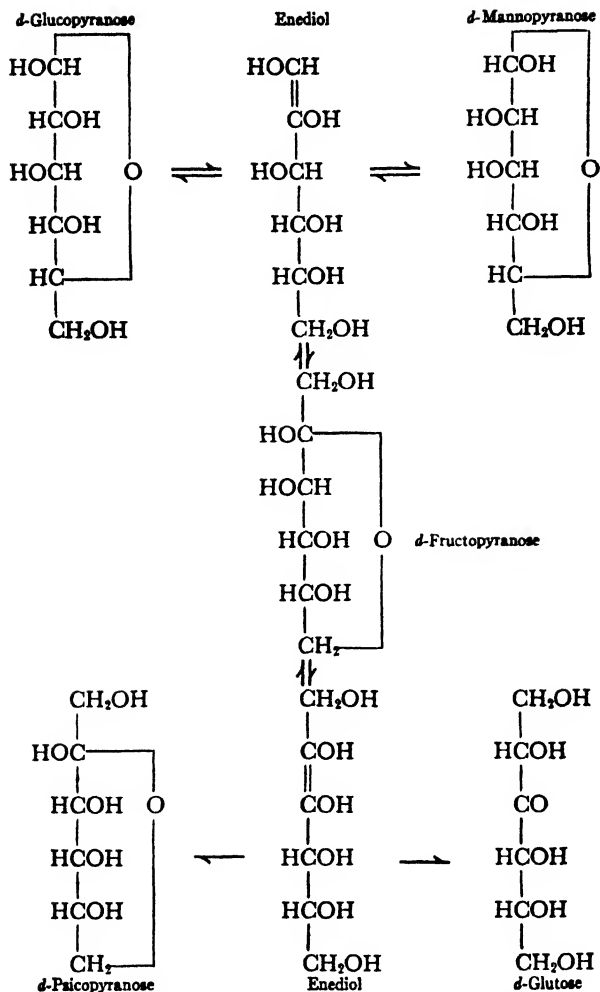
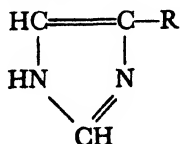


FIG. 5. Interconversion of *d*-glucose and related monosaccharides in alkaline solution.

Solutions of ammonia react with sugar degradation products to form imidazole compounds of the type:



These are related to the amino acid, histidine.

saccharides, and polysaccharides. Hence, the color tests are given by reducing and non-reducing carbohydrates.

The *Molisch test* is a very sensitive general reaction for carbohydrates. It is performed by adding a few drops of alcoholic α -naphthol solution to an aqueous sugar solution, and stratifying the mixture above concentrated sulfuric acid. Under these circumstances, furfural is liberated from most sugars and a characteristic red or violet ring appears; keto-1-uronic acids give green colors. The sugar alcohols, carboxylic acids, amino sugars, dihydroxyacetone, and certain keturonic acids give no color in this test.

In *Seliwanoff's test*, the sugar is boiled for thirty seconds with a dilute solution of resorcinol in 12 per cent hydrochloric acid; an orange to red color appears when a ketose is present. The *Tashiro-Tietz test* is a reverse Pettenkoffer reaction; it is performed by mixing the dilute sugar solution with an equal volume of freshly prepared bile salt in 1 : 1 sulfuric acid. In this test, ketoses give a violet color. While ketoses and certain aldohexoses and keturonic acids of the hepturonic series give the color tests, osones and most keturonic acids do not. Color tests for ketoses are only relatively specific; they require careful treatment with specified concentrations of acid, in order to avoid false responses due to the slow formation of hydroxymethylfurfural from aldohexoses.

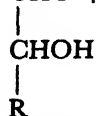
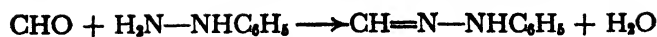
A sensitive reaction for pentoses and certain uronic acids is *Bial's test*, which is dependent on the production of furfural. A dilute orcinol-ferric chloride solution in 30 per cent hydrochloric acid is heated to boiling, and immediately after removing it from the flame several drops of the dilute sugar solution are added. A green color develops in the presence of trioses, methyltetroses, pentoses, digitoxose, aldohexuronic acids, or keto-6-uronic acids. Ketoheptoses give a purple coloration, while ketoses and methylpentoses produce orange-colored solutions which deposit dark green precipitates on standing. Phloroglucinol can be substituted for orcinol, in which case a red furfural conjugation product is formed.

The *naphthoresorcinol test* is used to detect common uronic acids. The sugar, in 1 N hydrochloric acid solution, is heated for five minutes on a boiling water bath with a small quantity of naphthoresorcinol. The pigments which are formed are then extracted with ether. The ethereal solution is colored pink, violet, or purple when uronic acids are present. Keto-6-uronic acids give an intense blue color in this test.

In the presence of concentrated hydrochloric acid, the 2-desoxy sugars impart a green color to a pine splint; in glacial acetic acid solution they give a blue color in the presence of ferrous salt and sulfuric acid (Kiliani's test). The determination of amino sugars is described on page 295.

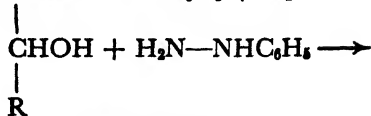
CONDENSATION OF REDUCING SUGARS WITH NITROGENOUS COMPOUNDS

Reducing sugars react with excess phenylhydrazine in dilute acetic acid solution to form osazones:

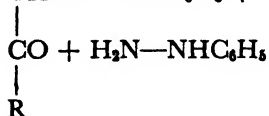
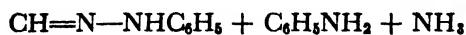


Aldose

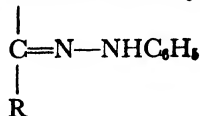
Phenylhydrazine



Aldose Phenylhydrazone



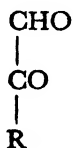
Osone Phenylhydrazone



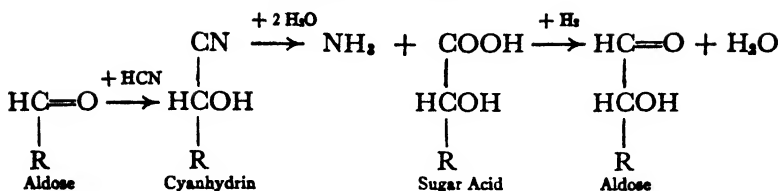
Phenyllosazone

Ketoses undergo similar reactions (in reverse order, starting at carbon 2). Aldose and ketose stereoisomers which have identical arrangements at all but the two carbon atoms involved in the reactions yield the same osazone; thus *d*-glucose, *d*-mannose, and *d*-fructose give phenylglucosazone. The 2-desoxy sugars form no osazones. Hydrazones can readily be produced at room temperature, but it is usually necessary to heat acidified sugar solutions with 3 to 4 mols of phenylhydrazine for thirty minutes or more in order to secure osazones. The phenylhydrazones exist in α - and β -modifications; they are quite soluble except in the case of *d*-mannose and *l*-fucose.

Osazones are insoluble yellow crystals whose form, melting point, and optical activity serve to identify the parent sugar (Table 48, page 268). The phenyllosazones of disaccharides are rather soluble. Other substituted hydrazines are frequently used in place of phenylhydrazine. Hydrazones can be decomposed by fuming hydrochloric acid, benzaldehyde, or formaldehyde yielding the original sugar. Similar treatment of osazones yields *osones*, which are dicarbonyl sugars of the general type:

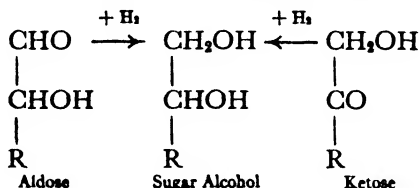


Reducing sugars also unite with hydroxylamine to form *oximes*, and with purines and pyrimidines to produce nucleosides (Table 55, page 287). In slightly alkaline solutions, hydrocyanic acid unites in C—C linkage with the reducing carbon of sugars to form *cyanhydrins*. These compounds may be hydrolyzed to sugar acids, and the latter reduced to sugars which contain one additional carbon atom, thus providing a method of carbohydrate synthesis:



SUGAR ALCOHOLS

The reduction of aldoses or ketoses, by sodium amalgam or by catalytic hydrogenation, produces the corresponding polyalcohols:

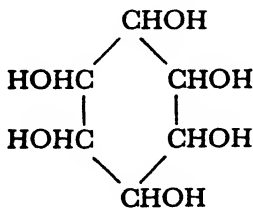


The reduction of a ketose gives a mixture of two isomeric alcohols inasmuch as an additional carbon atom becomes asymmetric.

The distribution of common sugar alcohols has been given in Table 49, page 272. These alcohols are used frequently in bacterial culture media. Formulae for the more important sugar alcohols may be found in Table 51. The sugar alcohols have only small specific rotations, but the rotations can be markedly increased by adding sodium borate or other coordinating salt. Erythritol and dulcitol are *meso* compounds (page 266). *d*-Sorbitol is formed from *d*-glucose, *d*-fructose, or *l*-sorbose; *d*-mannitol is formed from *d*-mannose or *d*-fructose; and dulcitol is formed from *d*-galactose, *l*-galactose, *d*-tagatose, or *l*-tagatose.

CYCLITOLS

These are cyclic polyalcohols which are derived, biologically, from sugar alcohols. The best known natural cyclitols are stereoisomeric *inositols* of the following structure:



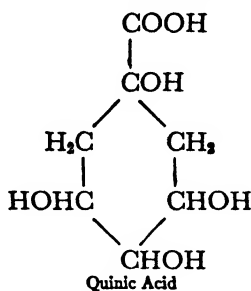
Both *d*- and *l*-inositols occur in plant resins; *meso* inositol is widely distributed, in the free form, in animal tissues, where it is also a component of the

TABLE 51

SUGAR ALCOHOLS

CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH	CH ₂ OH
CHOH	HCOH	HOCH	HCOH	HOCH	HCOH	HCOH
CH ₂ OH	HCOH	HCOH	HCOH	HOCH	HOCH	HOCH
	CH ₂ OH	HCOH	HCOH	HCOH	HCOH	HOCH
		CH ₂ OH	CH ₂ OH	HCOH	HCOH	HCOH
				CH ₂ OH	CH ₂ OH	CH ₂ OH
Glycerol	<i>meso</i> -Erythritol	<i>d</i> -Arabitol	<i>d</i> -Ribitol	<i>d</i> -Mannitol	<i>d</i> -Sorbitol	Dulcitol

cephalin known as lipositol (page 191). In plants it occurs principally as phytin (a calcium magnesium salt of inositol hexaphosphoric ester). Phytin is hydrolyzed by the plant enzyme, phytase; but it is not easily split by gastro-intestinal enzymes. Hence, its phosphoric acid radicals are unavailable for animal nutrition. Related to the cyclitols is the quinic acid of cranberries, plums, prunes, and coffee beans:



The ingestion of quinic acid by human beings causes an increased excretion of hippuric acid.

OXIDATION

Carbohydrates are most easily oxidized in neutral or in alkaline solutions; a sufficiently large concentration of hydrogen ions inhibits sugar oxidation. Anions are the active oxidants in analytical sugar reagents, as, for example, cupritartrate in copper reagents, dinitrosalicylate in Sumner's reagent, and ferricyanide in the Hagedorn-Jensen reagent. Hypobromite, hypoiodite, and periodate are also very useful reagents for sugar oxidation. These anions probably combine with carbohydrates in a manner analogous to that of the tungstates, molybdates, borates, carbonates, and hydroxides, which readily form coordination compounds with sugars in acid or in alkaline solutions. Addition reactions are dependent on the hydroxyl radicals of carbohydrates. They are the chief

reactions of the polysaccharides since these sugars do not have reducing radicals.

Solutions of glucose, or of the glucose-boric acid coordination compound, are oxidized rather slowly by bromine (hypobromite); but when certain cations are introduced as borates, tungstates, molybdates, carbonates, or hydroxides, carbohydrate oxidation is greatly accelerated even in slightly acid solution. Cations are therefore important catalysts for the oxidation of sugar. Iron, aluminum, copper, and silver are most active in this respect, but alkaline earth and alkali cations are also effective. Carbonates and bicarbonates of these cations markedly accelerate sugar oxidation in slightly alkaline, neutral, or slightly acid solutions.

The catalytic influence of alkalis on carbohydrate oxidation depends on the formation of coordination compounds (by hydroxides and carbonates), catalysis (by cations of the alkali), and neutralization of inhibitory acids formed during the oxidation.

ANALYTICAL REAGENTS

The reagents most widely employed for the detection and determination of reducing sugars are various alkaline copper solutions which contain carbonate, tartrate, salicylate, citrate, and the like. Benedict's qualitative copper reagent contains sufficient citrate to prevent the formation of a characteristic yellow to red precipitate of cuprous oxide except by abnormal urinary concentrations of sugar (above 0.1 per cent). Bertrand's reagent has been used extensively for industrial sugar analysis and the Folin-Wu¹ and Shaffer-Hartman reagents are used for the determination of blood sugar. Sugar solutions must be heated with quantitative oxidizing reagents under carefully standardized conditions; the reduced copper is estimated by titration, or (as in the Folin-Wu method) colorimetrically by means of phosphomolybdic acid. Alkaline cupritartrate reagents are reduced at room temperature by dioses, trioses, certain tetroses, dicarbonyl sugars, and keturonic acids which have less than seven carbon atoms.

The Hagedorn-Jensen method employs ferricyanide as the sugar oxidant; in Sumner's method, the oxidant is dinitrosalicylate. Of the useful sugar oxidants, dinitrosalicylate is least affected by nonsugar substances. Sumner's method is not sensitive enough for the determination of blood sugar, but it is particularly useful for the rapid colorimetric determination of urine sugar and for studies of pure sugars. This method gives nearly identical values for many ordinary monosaccharides, whereas copper reagents have different reducing values for the individual sugars (Table 48, page 268).

In the analysis of blood and other biological fluids, the proteins are first

¹ For exact determinations, the Folin-Wu reagent requires the following correction factors: (mg. per cent \times 0.88) + 12, for 0.1 mg. standards; and (mg. per cent \times 0.855) + 29, for 0.2 mg. standards.

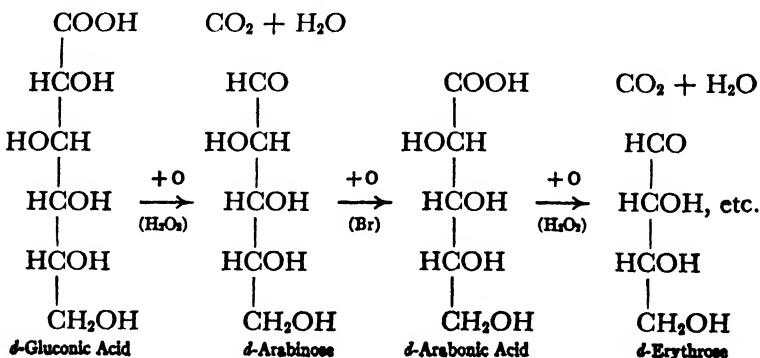
precipitated by tungstic acid, or other protein precipitant, and the filtrate is used for the determination of reducing sugar. The *d*-glucose fraction of the reducing material can be removed by fermenting with washed yeast; its concentration is equal to the difference between the original reducing value and the reducing value after fermentation.

ASYMMETRIC OXIDATION

Quantitative differences in the reduction of cupritartrate reagents by various sugars (Table 48, page 268) are partly traceable to the asymmetry of the oxidizing anions (copper complexes of *d*-tartaric acid) present in these reagents. The asymmetric anions resemble enzymes in their sensitivity to sugar asymmetry. Studies with *d*-, *l*-, and *meso*-cupritartrate reagents indicate that *cis-trans* arrangements and symmetric balancing in the sugar molecule exert important influences on sugar oxidation; *trans* or balanced molecules are most easily oxidized. *d*-Glucose is maximally *trans*, and is comparatively symmetrical in all dimensions; per mol, it reduces more copper than the other monosaccharides. Methylpentoses are unsymmetrical and reduce little copper, but they are very susceptible to other oxidants.

SUGAR ACIDS

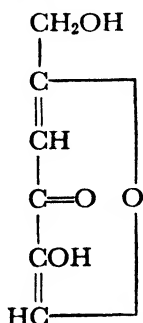
The oxidation processes considered above lead to the formation of sugar acids and their lactones. The primary oxidation product of an aldose is an equilibrium mixture of the corresponding *monocarboxylic acid* and its γ - and δ -lactones (Table 52). Acidity favors accumulation of the furanoid or γ -lactone, while alkalinity stabilizes the free acid. *d*-Glucose is oxidized to *d*-gluconic acid by certain bacteria and fungi, and animal tissues can oxidize the 6-phosphoric ester of glucose to 6-phospho-*d*-gluconic acid. Alternate oxidation by hydrogen peroxide and bromine degrades monocarboxylic acids by oxidative decarboxylation to a series of aldoses and acids with progressively shorter chains:



Oxidation by nitric acid converts monocarboxylic acids to *dicarboxylic acids* (Table 52); these are stable substances which tend to accumulate in oxidizing solutions. Mucic acid is formed by nitric acid oxidation of *d*-galactose and its derivatives; because of the marked insolubility of mucic acid, the reaction serves as a test for galactose.

Monocarboxylic acids can also be oxidized to *uronic acids* which are of considerable biological interest (for distribution, see Table 49, page 272). Uronic acids are reducing sugar acids which are readily decarboxylated in hot acid solution (page 276). They are classified as *alduronic* and *keturonic* acids. The former are produced by plant and animal tissues, and by hydrogen peroxide *in vitro*. Keturonic acids are formed by plants and the acetic bacteria, and by hydrogen peroxide or bromine *in vitro*. Bromine water oxidizes aldoses rapidly and ketoses very slowly; it also serves as a reagent for differentiating alduronic and keturonic acids. Formulae for several uronic acids are given in Table 52. The keturonic acids are very reactive compounds which enolize rapidly, and which reduce analytical reagents at room temperature. They are easily destroyed by acids, alkalis, and oxidizing agents. Keturonic acids which have the carbonyl radical adjacent to the carboxyl radical are *osonic* acids, while those having the carbonyl radical at the other penultimate carbon are termed *onosic* acids.

l-Ascorbic acid, or vitamin C, is the enolic form of a keturonic acid lactone (Table 52). This vitamin has a stable lactone ring; its acidity is due to ionization of hydrogen at carbon 2 or 3. Ascorbic acid and its reversible oxidation product, dehydroascorbic acid, constitute an important biological oxidation-reduction system (page 105). Ascorbic acid is determined, quantitatively, by titrating metaphosphoric or trichloroacetic acid filtrates of biological fluids with 2,6-dichlorophenolindophenol, a blue indicator which accepts two hydrogens from ascorbic acid to form dehydroascorbic acid and the colorless leuco base of the indicator. Ascorbic acid in plant foods is destroyed readily by heating, boiling, or even by prolonged exposure to the air. Hence, aged or stored foods frequently contain

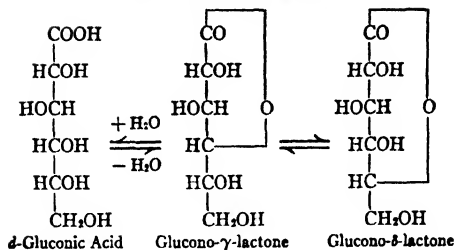


Kojic Acid (See page 286.)

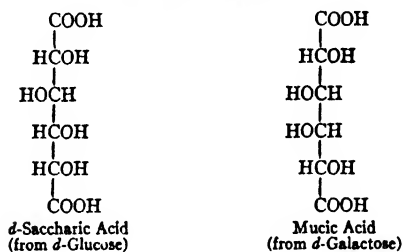
TABLE 52

SUGAR ACIDS

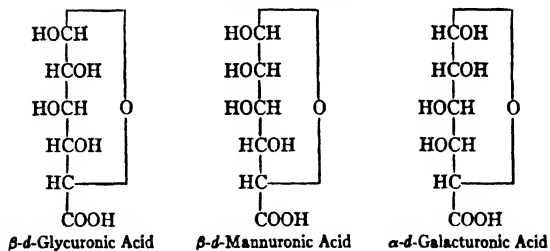
MONOCARBOXYLIC ACIDS



DICARBOXYLIC ACIDS



ALDURONIC ACIDS



KETURONIC ACIDS

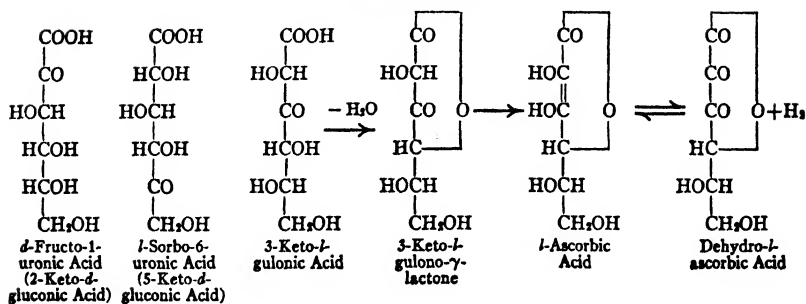
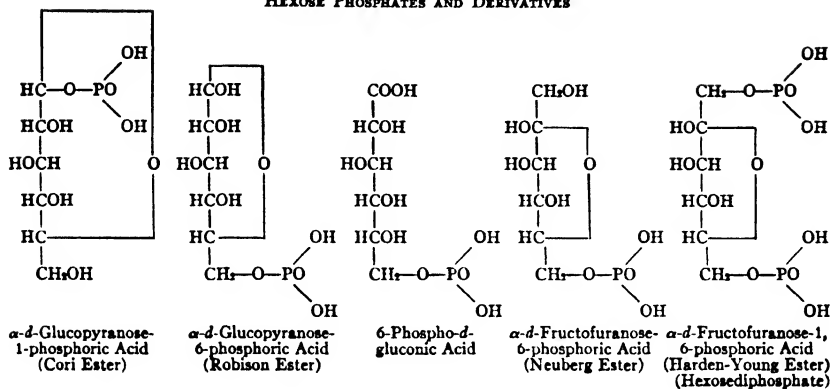


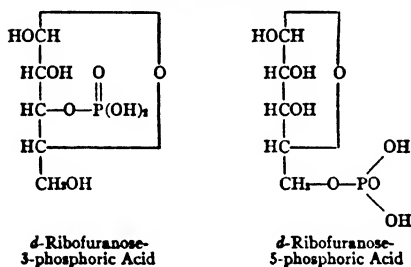
TABLE 53

PHOSPHATE ESTERS OF SUGARS

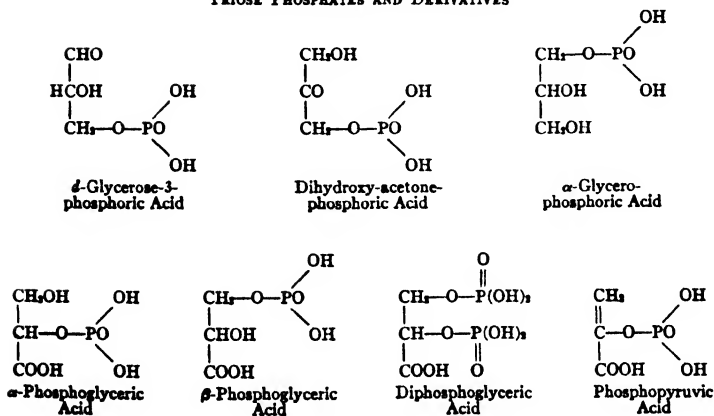
HEXOSE PHOSPHATES AND DERIVATIVES



PENTOSE PHOSPHATES



TRIOSE PHOSPHATES AND DERIVATIVES



little of the vitamin. The international unit of vitamin C is equal to 0.05 mg. of *l*-ascorbic acid (page 665).

Certain acetic bacteria and fungi convert fructose and other carbohydrates to *kojic acid*, an enolic pyrone derivative, which is related chemically to antibiotic agents (page 481). (See page 283 for formula.)

SUGAR ESTERS

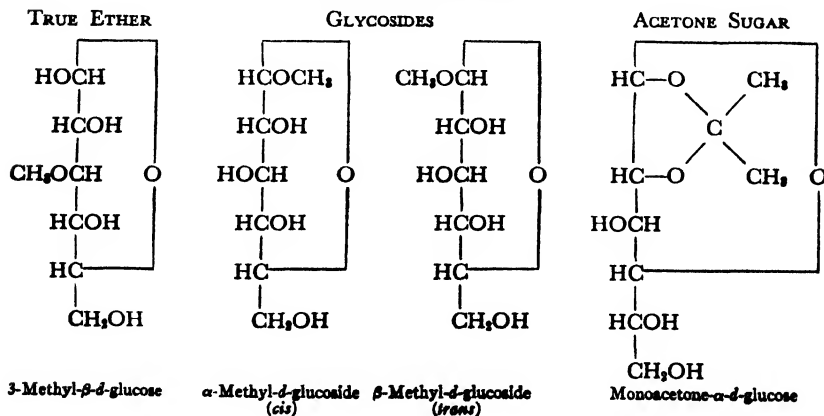
The hydroxyl radicals of carbohydrates are easily esterified with acids. The acetates, benzoates, and carbonates of sugars are rather insoluble crystalline esters which are used in studies of sugar structure. The naturally occurring esters include the sulfate and phosphate esters, the gallotannins or gallic acid esters, and the crocins (page 207). Triose and hexose phosphates are important metabolic intermediates in plant and animal tissues. Formulae for the principal sugar phosphates are given in Table 53. These esters are readily hydrolyzed by acids and by phosphatases. Sugar phosphate esters are stronger acids than phosphoric acid (Table 1, page 4). The naturally occurring α -glyceophosphoric acid is the *l*-form. *d*-Ribose-3-phosphoric acid is a unit of plant nucleic acids, and *d*-ribose-5-phosphoric acid is found in the nucleotides of animal tissues. Glycogen, starch, and trehalose exist in animal and plant tissues partially esterified as 6-phosphoric esters. Sulfuric acid esters of carbohydrates are components of the polymeric heparins, sulfomucins, and agar-agar.

SUGAR ETHERS

These carbohydrate derivatives have an organic radical, called the *aglycone*, united to the sugar through an oxygen bridge; they are hydrolyzed

TABLE 54

SUGAR ETHERS



by acids but not by alkalis. When the union is at the reducing carbon, the resulting acetal is termed a glycoside (Table 54). Methyl ethers (methylated sugars) are easily crystallized and distilled, and have been widely used in determinations of carbohydrate structure. Sugars which have *cis* hydroxyls can unite with acetone to produce heterocyclic "acetone sugars" of the type shown in Table 54; since these are furanoid substances, they may be hydrolyzed by acids to prepare furanose derivatives.

GLYCOSIDES

The term glycoside is often applied indiscriminately to natural compounds which yield sugar on hydrolysis. True glycosides contain aglycone radicals such as alcohol, phenol, sterol, isothiocyanate, purine, or pyrimidine at the reducing carbon of the sugar. Generally, glycosides do not reduce, do not form osazones, and, with the exception of the N-glycosides, do not mutarotate. *trans* glycosides are usually hydrolyzed more easily *in vitro* than are *cis* glycosides; and enzymes are susceptible to *cis-trans* arrangement of the glycoside linkages. Furanoid, septanoid, and 2-desoxy glycosides are hydrolyzed very rapidly, but the nature of the aglycone

TABLE 55

TYPE	GLYCOSIDES	EXAMPLES
Alkyl glycosides . . .	Synthetic methyl and ethyl ethers	
Phenol glycosides . . .	Arbutin, salicin, phlorhizin, in plants (drugs)	
Glycuronides . . .	The paired or conjugated glycuronates formed as detoxication products of alcohols, sterides, and phenols, in animals	
Cerebrosides . . .	Sphingosine galactoside and glucoside derivatives, in animals	
Phytosterolins . . .	Sterol glycosides of plants	
Cardiac glycosides . .	Steride glycosides of digitoxose, etc., in plants (drugs)	
Saponins	Glycosides of steride sapogenins, in plants	
Anthracene glycosides .	Glycosides of alizarin aglycones, in plants (drugs)	
Anthocyanins	Plant sap pigments (glycosides of anthocyanidins and glucose, galactose, rhamnose, pentoses, or bioses)	
Anthoxanthins	Plant sap pigments chemically related to anthocyanins (glycosides of flavones and flavonols) ¹	
Cyanophoric glycosides .	Glycosides of hydroxynitriles; amygdalin, prunasin, in plants (drugs)	
S-Glycosides	Glycosides of mustard oils; sinigrin, sinalbin. (These glycosides of plants have sulfur bridges.)	
N-Glycosides	Nucleic acids, nucleotides, nucleosides, in animals and plants. (These glycosides have nitrogen bridges that are difficultly hydrolyzed.)	

¹ Flavonol pigments can act as gynotermones in flagellates.

radical affects hydrolytic rates. Natural *trans* glycosides of a variety of sugars are widely distributed in plants, and many are medicinal compounds. Important classes of glycosides are given in Table 55.

OLIGOSACCHARIDES

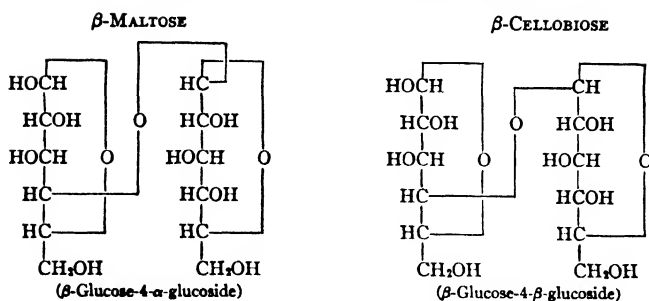
The distribution of oligosaccharides, and the enzymes which hydrolyze them, will be found in Table 57, page 291. The *disaccharides* or *bioses* may be regarded as glycosides in which a second molecule of sugar has replaced the aglycone. Formulae and chemical names of common disaccharides are given in Table 56. In natural reducing disaccharides, the sugar corresponding to the aglycone is frequently united at carbon 4, although the 6-linkage is also found. Maltose, lactose, cellobiose, and aldobionic acids are reducing sugars; cane sugar (sucrose) and trehalose, in which the potential reducing radicals participate in the glycoside linkage, do not reduce analytical reagents. Disaccharides can be hydrolyzed to the component monosaccharides by acids and by enzymes (glycosidases).

Furanoid oligosaccharides, such as sucrose, melezitose and raffinose, are hydrolyzed by acids a thousand times as rapidly as are the pyranoid glycosides. The hydrolysis of sucrose is sometimes termed inversion, inasmuch as the optical rotation changes from right to left as the *d*-glucose and *d*-fructofuranose are liberated. The latter is a labile monosaccharide which changes rapidly to ordinary *d*-fructopyranose. The pyranoid *cis* disaccharides (maltose, trehalose) are hydrolyzed less readily than are the corresponding *trans* disaccharides (cellobiose, lactose), but *trans* aldobionic acids (β -uronides) are hydrolyzed slowly.

POLYSACCHARIDES

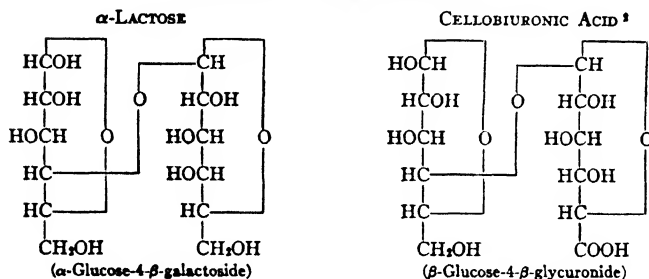
These are polymeric carbohydrates with chain structures and colloidal properties. Many form gels, particularly those which contain uronic, phosphoric, or sulfuric acid radicals. Polysaccharides may be considered as polymers of anhydro sugars. High molecular sugars appear to be produced in nature by the formation of glycoside linkages; the long glycoside chains then unite through the secondary or accessory valences of their oxygen atoms (especially those of ether linkages) to produce colloidal aggregates or micelles. Polysaccharides are non-reducing since all of the potential reducing radicals (except a very few "end groups") are in glycoside linkage. These carbohydrates are structural and storage materials of animals and plants. They are hydrolyzed by acids, but not by alkalis. *Simple* or homogeneous polysaccharides contain only one type of monosaccharide unit, while *complex* or mixed polysaccharides have several different units. The distribution, structures, and minimal molecular weights of polysaccharides, together with the names of the enzymes which hydrolyze them, are summarized in Table 57. Molecular weights deter-

TABLE 56

REDUCING DISACCHARIDES ¹

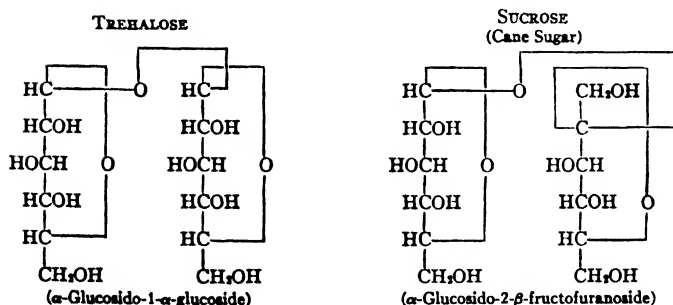
Gentiobiose is β-Glucose-6-β-glucoside.

Melibiose is β-Glucose-6-α-glucoside.



The *aldobionic acid* of gum acacia is α-Galactose-6-β-glycuronide.

NON-REDUCING DISACCHARIDES



NON-REDUCING TRISACCHARIDES

Raffinose is 2-β-Fructofuranosido-1-α-glucosido-6-α-galactoside.

Melissiose is 1-α-Glucosido-2-β-fructofuranosido-6-α-glucoside.

¹ In these formulae, the α-glucoside linkages are *cis* linkages and the β-glucoside linkages are *trans* linkages.
² The aldobionic acid of *Pneumococcus* Types II, III, and VIII. In the Type III pneumococcal polysaccharide, the cellobiuronic acid units are united with each other by 1,3 linkages.

mined by the ultracentrifuge are much higher than the values reported in the table.

Simple Polysaccharides

X-ray analysis has shown that simple polysaccharides consist of long open chains. The number of monosaccharide units varies greatly; for example, certain crystalline dextrans contain only five units, whereas in some polysaccharides there are thousands of units. Photographic observation of the velocity of sedimentation in the ultracentrifuge (Svedberg's method) indicates that a native *cellulose* micelle can have a molecular weight of 1,000,000 to 1,840,000, which corresponds to 6,200 to 11,300 anhydroglucose units. Such molecular bundles of unbranched chains unite to form the cellulose fibers. Cellulose is the most abundant of organic compounds; the equivalent of a third of the carbon dioxide present in the earth's atmosphere (about seven hundred billion kilograms) is anchored as cellulose.

It is believed that *starch* and *glycogen* have branched chains. Starch is a mixture of homologues of different molecular weights (10,000 to 1,000,000). The chief fractions are soluble amylose (unbranched chain) and insoluble amylopectin (branched chain); the latter has 1,6 linkages at the branching points. β -Amylase hydrolyzes the amylose fraction of starch almost quantitatively to maltose, in contrast to 50 per cent conversion of glycogen or of the amylopectin fraction of starch. Amylose constitutes 17 to 34 per cent of the various starches; its chain contains about 300 anhydroglucose units, while the branches of the amylopectin molecule have approximately 25 units each. Glycogen also has a branched chain. Sedimentation in the ultracentrifuge indicates a molecular weight of approximately 4,000,000 for the glycogen aggregates, with almost 25,000 anhydroglucose units in the micelle. Both glycogen and starch exist partly as phosphoric acid esters, and they are both hydrolyzed to dextrans, maltose, and *d*-glucose. Starch and glycogen give blue and red-brown colors, respectively, with dilute iodine solutions. The colorations of these adsorption compounds disappear on warming and reappear on cooling.

The *dextrans* are mixtures of low molecular polysaccharides which are produced by the slight hydrolysis of glucosans. In the order of decreasing molecular weights, the dextrans are classified roughly as soluble starches (amylodextrans), erythrodextrans, and achroodextrans. These give blue, red, and colorless solutions, respectively, in the iodine test. One of the best characterized dextrans is the limit dextrin produced during the hydrolysis of starch by amylases. Dextrin and *inulin* preparations have detectable reducing radicals; these react especially with Sumner's reagent. Because of its furanoid structure, inulin is rapidly hydrolyzed by acids. *Levan* particles have molecular weights of 50,000,000 to 100,000,000, according to sedimentation experiments.

Partial hydrolysis of *chitin* gives chitobiose, a disaccharide consisting of

TABLE 57
OLIGOSACCHARIDES AND POLYSACCHARIDES OF BIOCHEMICAL INTEREST

OLIGOSACCHARIDES	GLYCOSIDE STRUCTURE ¹		HYDROLYZED BY	DISTRIBUTION IN NATURE
	Linkage	Number		
<i>Disaccharides</i> ($C_{12}H_{22}O_{11}$)				
Maltose	1,4- α -Glucoside		α -Glucosidase ²	Hydrolytic product of starch, dextrans, and glycogen
Cellobiose	1,4- β -Glucoside		β -Glucosidase ³	Hydrolytic product of cellulose
Lactose (milk sugar)	1,4- β -Galactoside		Lactase ⁴	In milk
Aldobionic acids	Uronides			Units of gums and prosthetic polysaccharides
Trehalose	1,1- α , α -Glucoside		Trehalase	In marine algae, bacteria, fungi, and yeasts
Sucrose (cane sugar)	1- α -Glucosido-2- β -fructofuranoside		Invertase, α -glucosidase	Reserve food in plants
<i>Trisaccharides</i> ($C_{18}H_{32}O_{16}$)				
Raffinose	6- α -Galactosido-1- α -glucosido-2- β -fructofuranoside		Invertase, α -galactosidase	Accompanies sucrose in plants
Melezitose	1- α -Glucosido-2- β -fructofuranosido-6- α -glucoside		α -Glucosidase	In gummy exudates of plants
<i>SIMPLE POLYSACCHARIDES</i>				
			APPROX. MOL. WT. ⁵	DISTRIBUTION IN NATURE
<i>Pentosans</i> ($C_5H_8O_4$) _n				
Xylans ⁶	1,4- β -Xyloside	16-20	Xylanase ⁷	In cell walls of plants
<i>Hexosans</i> ($C_6H_{10}O_5$) _n				
Cellulose	1,4- β -Glucoside	200-250	Cellulase ⁸	Fibrous constituent of plants, tunicates, and bacteria

Mucilages	Aldobionic acids, ¹⁵ etc.	In seeds
Agar-agar	1,3- <i>d</i> -Galactoside, <i>l</i> -galactose, sulfuric acid ¹⁸	In marine algae
<i>Prosthetic polysaccharides</i>		Widely distributed in living cells as glycoproteins
<i>Animal polysaccharides</i>		
Neutral polysaccharides	N-Acetyl- <i>d</i> -glucosamine, <i>d</i> -mannose, <i>d</i> -galactose, <i>d</i> -glucose	Prosthetic radicals of mucoids; blood group haptens
Acid polysaccharides	N-Acetyl- <i>d</i> -glucosamine, <i>d</i> -glycuronic acid	Prosthetic radicals of mucins
Hyaluronic acid ¹⁶		Prosthetic radicals of sulfomucins
Polysaccharide-sulfuric esters		
Mucoitin-sulfuric acid	N-Acetyl- <i>d</i> -glucosamine, <i>d</i> -glycuronic acid, sulfuric acid	Prosthetic radicals of mucoproteins; heparins
Chondroitin-sulfuric acid	N-Acetyl- <i>d</i> -galactosamine, <i>d</i> -glycuronic acid, sulfuric acid	Prosthetic radicals of chondroproteins
<i>Bacterial polysaccharides</i> ¹⁷	N-Acetyl- <i>d</i> -glucosamine, aldobionic acids and various monosaccharides	Prosthetic radicals of bacterial proteins (haptens of bacterial antigens); also in gummy capsules and gelatinous secretions of bacteria

¹ Unless otherwise noted, these are pyranoid forms.

² Commonly called maltase.

³ Commonly called emulsin.

⁴ Lactase is probably a β -glucosidase.

⁵ Calculated from chemical data; ultracentrifuge gives much higher values.

⁶ Xylans are actually complex polysaccharides with at least one *l*-arabinose unit.

⁷ Found in bacteria and plants.

⁸ Found in bacteria, fungi, protozoa, crustacea, and mollusks.

⁹ Found in plants.

¹⁰ Found in snails.

¹¹ Pectins are methyl esters of pectic acid. The latter contains 8 to 10 *l*,4-*d*-galactose unit whose carbon 6 is esterified with the sulfuric acid.

galacturonic acid units and is hydrolyzed by the enzyme, pectinase, of plants and the Granulobacter group of bacteria.

¹² Found in bacteria, fungi, plants.

¹³ Hydrolyzed by cytaes of fungi.

¹⁴ This aldobionic acid is α -galactose-6- β -glucuronide. The aldobionic acid units are joined as a galactose chain; the arabinose is attached at carbon 4 of the glycuronic acid units.

¹⁵ One of these is *l*-thiamnose-2-galacturonide.

¹⁶ Hydrolyzed by hyaluronidase of animal tissues and bacteria.

¹⁷ See page 473 for detailed information.

¹⁸ The agar chains contain about 9 *d*-galactoside units terminated by a *l*,4-*d*-galactose unit whose carbon 6 is esterified with the sulfuric acid.

two molecules of *d*-glucosamine; chitin is hydrolyzed with difficulty, to acetic acid and *d*-glucosamine. Chitin and cellulose are very insoluble polysaccharides which dissolve only in concentrated acids. Cellulose dissolves also in cuprammonium hydroxide solutions. Xylans and mannans are alkali-soluble. Starch grains disintegrate in hot water to form colloidal pastes. Glycogen, the dextrins, and inulin are all water-soluble.

Complex Polysaccharides

These polymers contain several different monosaccharide units, one of which is usually a uronic acid. The *hemicellulose* polyuronides are alkali-soluble constituents of the cell walls of plants; they are divided into two general groups, as shown in Table 57. Hemicelluloses are hydrolyzed very slowly by bacteria. They form the major part of the indigestible residue or fiber of foods. Other complex polysaccharides may be conveniently designated as polysaccharide acids. They differ from hemicelluloses in that they have free uronic carboxyl radicals which can readily combine with proteins and cations. *Pectins* are the substances responsible for the jellying properties of preserves and jellies. The soluble pectins of fruits are inorganic salts of pectic acid methyl esters. Their molecular weight, as determined by the ultracentrifuge, is from 25,000 to 50,000. Gums and mucilages are similar acidic plant polyuronides; gum acacia is a familiar example (Table 57). It has a molecular weight of approximately 290,000 as determined by the ultracentrifuge.

Prosthetic Polysaccharides of Glycoproteins

Since they contain prosthetic polysaccharide radicals, many animal and bacterial proteins give positive Molisch tests (Table 57). Some of these polysaccharides also exist in uncombined form in cells and tissues. The blood group A hapten is a neutral prosthetic polysaccharide, which has a molecular weight of approximately 10,000. The most common structural unit of prosthetic polysaccharides is N-acetyl-*d*-glucosamine.

Mucoid proteins of animals contain neutral polysaccharides; small quantities of such polysaccharides are present in certain albumins, globulins, caseins, and collagens. Thrombin, prothrombin, the gonadotropic hormones of the anterior pituitary lobe, and the chorionic gonadotropic hormones contain sufficient neutral polysaccharide to be considered as mucoids.

The mucins and sulfomucins are protein salts of acid polysaccharides. The mucins contain hyaluronic acid, while the sulfomucins have sulfuric acid esters of polysaccharides. The sulfomucins (formerly termed mucins) are classified as mucoproteins and chondroproteins; they contain mucoitin-sulfuric and chondroitin-sulfuric acids, respectively. The natural heparins are mucoitin-sulfuric acid polysaccharides. Mucins and mucoproteins occur in mucous secretions (including synovial and ocular fluids), also in

connective tissue, the cornea, the umbilical cord, and so forth. The chondroproteins are structural substances of bone, cartilage, sclera and tendon. The structural units of the acid prosthetic polysaccharides are listed in Table 57; a classification of the glycoproteins is given in Table 70, page 391. Note that mucoitin-sulfuric acid and chondroitin-sulfuric acid have different amino sugar units. The molecular weights of chondroitin-sulfuric and hyaluronic acids are approximately 200,000 and 200,000 to 400,000, respectively.

The bacterial polysaccharides exist in free form, as constituents of waxes, and as protein compounds (complete bacterial antigens). These polysaccharides act as haptens, and determine the strain characteristics of bacteria; they are found in largest quantity in virulent smooth forms of bacteria. A few bacterial polysaccharides (cellulose, dextrans, and levans) are simple polysaccharides; but those of immunological interest are complex polysaccharides which contain not only acetylglucosamine, aldobionic acids, and the other units of the animal prosthetic polysaccharides, but also *d*-arabinose, *l*-rhamnose, inositol, and so forth. The carbohydrate units of type-specific pneumococcal polysaccharides are listed in Table 80, page 472. The composition and serological relations of bacterial polysaccharides are discussed on pages 471 to 474.

Determination of Polysaccharides

Polysaccharides are usually determined by hydrolysis with acid, and estimation of the reducing value of the liberated monosaccharides. Tissue glycogen is markedly resistant to alkali; it can, therefore, be separated from tissues by decomposing them with boiling alkali, filtering, and precipitating glycogen by the addition of alcohol. The glycogen is then hydrolyzed, and its reducing value determined. With the exception of glucosamine, the monosaccharide units of polysaccharides can be determined by treating the polysaccharide with concentrated acid and either tryptophane or orcinol; these reagents conjugate with the liberated furfural to form colored compounds which are then determined in the step photometer. The glucosamine content of polysaccharides is determined by warming the hydrolyzed sugar with acetylacetone in sodium carbonate solution. The pyrrole derivatives thus formed are conjugated with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) and determined colorimetrically.

METABOLISM

"New truth, like new beauty, cannot be totally unlike the old; and hence, the proper use of old knowledge is an indispensable aid to discovery of the new." — MORRIS R. COHEN

FOOD CARBOHYDRATES

Carbohydrates constitute about two thirds of the caloric intake of human adults; this corresponds to the daily ingestion of approximately 450 gm.

of digestible carbohydrate. Wheat, corn, rice, rye, and other cereals contribute approximately 55 per cent, refined sugars 25 per cent, vegetables 10 per cent, fruit 5 per cent, and dairy products 5 per cent of the dietary carbohydrates of Americans. Starch is provided principally by cereals and vegetables; sucrose by fruits, vegetables, prepared sweet foods, and table sugar; and lactose by milk and dairy products. Small amounts of glucose are present in honey, fruits, and prepared sweet foods; fructose in fruits and honey; and dextrins and maltose in toasted or partially hydrolyzed cereal products, including the infant foods and glucose syrups (Karo). Fruits and vegetables also provide such carbohydrate derivatives as sugar alcohols, and ascorbic, citric, malic, and tartaric acids. A number of the rare sugars, which are present in foods in small quantities, are not utilized by animals. Indigestible polysaccharides are of interest because of their effects on the large intestine. The approximate carbohydrate content of common foods is given in Table 58.

TABLE 58

APPROXIMATE DIGESTIBLE CARBOHYDRATE OF
COMMON FOODS ¹

	PER CENT CARBOHYDRATE
Cane sugar	100
Cornstarch	90
Dates, honey, jellies	80
Breakfast cereals (dry), corn meal, corn syrup, flour, raisins	75
Crackers	70
Cakes	60
Bread, chocolate, sweetened condensed milk	50
Pies	40
Figs (dry), puddings, sweet potatoes	25
Bananas, beans (shelled), corn, potatoes, rice, prunes	20
Cherries, grape juice, ice cream, macaroni, pears, peas, pecans, tapioca	15
Blackberries, breakfast cereals (cooked), cocoa beverage, cranberries,	
grapes, evaporated milk, oatmeal, onions, pineapple	10
Apples, avocados, carrots, peaches	8
Beets, human milk	7
Grapefruit, lemons, oranges, orange juice, peanuts, plums, pumpkins,	
squash, string beans, walnuts	6
Apricots, buttermilk, cantaloupe, cocoanut, cow's milk, soups, straw-	
berries	5
Broccoli, cheese, cream, cucumbers, oysters, turnips	4
Asparagus, cabbage, cauliflower, egg plant, lettuce, okra, olives, rasp-	
berries, rhubarb, sauerkraut, tomatoes, watermelon	3
Lemon juice, liver, radishes, spinach	2
Celery	1
Butter, eggs, gelatin, fish, meats, salad oils	0-1

¹ Values for vegetables and cereals apply to cooked or boiled foods, except as noted.

Photosynthesis in Plants

The various carbohydrates of plants have often been considered as originating from *d*-glucose; the pentoses are supposed to arise from hexoses by oxidative decarboxylation. *d*-Glucose and, perhaps, other hexoses are synthesized by the green plant tissues from atmospheric carbon dioxide. This gas is inspired by the tissues, reduced to carboxyl radicals of organic acids (as shown by studies with radioactive carbon dioxide), and converted into sugar. Energy for the reduction is provided through the absorption of light by chlorophyll pigments. Formulae for chlorophyll *a* and chlorophyll *b* are given in Table 90, page 527; chlorophyll *b* occurs in largest amounts in plants which synthesize starch. These pigments are complex porphyrin derivatives; they resemble the heme pigments of animals but contain magnesium in place of iron. Chlorophyll donates hydrogen for an enzymatic reduction of carbon dioxide. Radiant energy regenerates the original pigment by the reduction of dehydrochlorophyll; oxygen, equivalent to the carbon dioxide of the first reaction, is liberated from water by dehydrogenation, and exhaled. The autotrophic green and purple bacteria contain bacteriochlorophyll; reduction of carbon dioxide by these organisms is dependent on the presence of oxidizable inorganic sulfur compounds. Other autotrophic bacteria can use the energy derived from the oxidation of ammonia, nitrite, or hydrogen sulfide for the synthesis of organic acids and carbohydrates from carbon dioxide without the intervention of chlorophyll. In fact, the reduction of carbon dioxide to form methane, acetic acid, formic acid, lactic acid, succinic acid, and similar compounds, without the assistance of radiant energy, is a very general bacterial metabolic phenomenon. Such fixation of carbon dioxide as carboxyl radicals of acids is inhibited by fluoride and iodoacetate, but not by thiamin deficiency. It has also been demonstrated that radioactive C¹⁴ ingested as sodium bicarbonate is partly deposited as hepatic glycogen in rats. Hence, the photosynthetic reduction of carbon dioxide by plants is a specialized adaptation of a fixation reaction common to many cells, and the particular role of light is concerned with the dehydrogenation of water to form oxygen.

CARBOHYDRASES

Enzymes which hydrolyze glycosides, oligosaccharides, and polysaccharides have marked specificities. A carbohydrase requires a certain local pattern in the molecule of its substrate. Two important and widely distributed enzymes which hydrolyze pyranosides have, in the past, been termed emulsin and maltase. Emulsin is actually a mixed enzyme preparation from bitter almonds. Its principal component, a β -glucosidase, is also found in other plants, animals, and fungi. This enzyme hydrolyzes all β -glucosides and *trans* pyranoid glycosides (*i.e.*, *trans* arrangement at the glycoside linkage). It would, therefore, be designated more correctly as

trans-pyranosidase. In plants, animals, and yeasts there is an α -glucosidase (maltase) whose hydrolytic action is restricted to α -glucosides that are not substituted at carbon 6, and to α -xylosides. α -Galactosidase and α -mannosidase are individual specific enzymes; the former hydrolyzes α -forms of *d*-galactosides and *l*-arabinosides, and the latter is hydrolytic to α -*d*-lyxosides and 2-desoxyglycosides as well as α -*d*-mannosides. Glycuronidases are regarded as separate enzymes. β -Galactosidase (lactase) is probably identical with β -glucosidase. *Invertase* (sucrase) of plants and yeasts hydrolyzes only β -fructofuranosides such as sucrose, raffinose, and inulin; the invertase of fungi is actually α -glucosidase. (For other carbohydrases, see Table 57, page 291.)

Enzymes which hydrolyze polysaccharides are very specific and have nothing in common with the glycosidases just described. The amylases of plants, animals, and bacteria hydrolyze starch, glycogen, and dextrins. They are classified as α - and β -amylases, which hydrolyze preferentially interior and end linkages, respectively. Ptyalin of saliva is chiefly α -amylase. Amylopsin of pancreatic juice is a β -amylase, and malt amylase is a mixture. Both ptyalin and amylopsin are activated by chloride anions. Most amylases require an additional unidentified coenzyme for the quantitative hydrolysis of starch or glycogen to maltose. In the absence of this coenzyme only 80 per cent of the theoretical quantity of maltose is formed, together with limit dextrin. The latter is formed from amylopectin and glycogen; amylose, with its unbranched chain, is converted quantitatively to maltose. The fragmentation of glycogen by internal animal tissues is not due to amylases, but involves the action of a phosphorylase and its coenzymes, adenylic acid and magnesium cations. This metabolic breakdown of glycogen is considered on page 321. Under physiological conditions, fragmentation of polysaccharides by phosphorylase systems is reversible, whereas the hydrolytic action of amylases is largely irreversible.

SALIVARY DIGESTION

The salivary amylase, ptyalin, rapidly hydrolyzes cooked starch, glycogen, and dextrin to the disaccharide, maltose. Only small quantities of maltose are formed during the short time in which the food remains in the oral cavity, but ptyalin continues to act up to one half hour in the food masses which accumulate in the stomach. When these masses are permeated by gastric juice, the lowered pH stops the action of ptyalin (optimum pH, 6.6). Less than two thirds of the food starch is thus hydrolyzed in the gastric cavity. Sucrose is partially split by the hydrochloric acid of gastric juice, but further gastric digestion of carbohydrates is impossible because of the lack of carbohydrases in gastric juice (Table 27, page 132.)

INTESTINAL DIGESTION

Ptyalin is absent from the saliva of many species of animals, and this amylase is quite unnecessary for the digestion of starch. The amylopsin of the pancreatic juice is a much more active amylase. It appears in normal pancreatic juice, after the first few weeks of infant life. Amylopsin hydrolyzes starch, glycogen, and dextrans to maltose which is then hydrolyzed to two molecules of *d*-glucose by α -glucosidase (maltase) of the pancreatic and intestinal juices. Lactose or milk sugar is hydrolyzed to *d*-glucose and *d*-galactose by lactase (β -glucosidase) of the succus entericus, while sucrose is hydrolyzed to *d*-glucose and *d*-fructose by invertase of the small intestine. The action of lactase is definitely slower than that of maltase or of invertase. These three carbohydrases are found in the succus entericus, and also intracellularly in the intestinal mucosa. Assuming that an adult ingests 1 quart of milk (40 gm. of lactose), 60 gm. of cane sugar, and 350 gm. of starch, the carbohydrate digestion products will be 20 gm. of *d*-galactose, 30 gm. of *d*-fructose, and 435 gm. of *d*-glucose daily.

BACTERIAL FERMENTATION

Pentosans, cellulose, pectins, gums, and hemicellulose polyuronides are not appreciably digested by the gastro-intestinal enzymes of mammals; but, with the exception of hemicelluloses and agar-agar, the polysaccharides are easily digested and fermented by intestinal bacteria and protozoa. The resulting fermentation products include acids (formic, acetic, propionic, butyric, lactic, and succinic), alcohols (ethyl, isopropyl, and butyl), and gases (hydrogen, methane, and carbon dioxide). Methane and hydrogen are formed by further fermentation of the acids; as stated previously, methane is a reduction product of carbon dioxide. The 4-carbon acids and alcohols are formed by condensation of acetaldehyde or pyruvic acid; these two substances and lactic acid are intermediate carbohydrate metabolites of living cells (Fig. 7, page 316).

The acids and alcohols produced in the intestine by bacterial fermentation are partially absorbed and utilized by animals. In man, they furnish relatively little energy; but herbivorous animals, which harbor microorganisms in their stomach pouches and intestines, utilize pentosans and celluloses as major foods. Although the human intestine contains cellulose-destroying organisms (*B. cellulosa* *dissolvens* and several fungi), the bacterial fermentation of cellulose and pentosans is incomplete in man, and variable amounts of these polysaccharides escape in the feces together with the hemicellulose polyuronides. About 80 per cent of the cellulose and pentosans of carrots and potatoes are fermented in the normal human intestine, and these foods leave only small fecal residues. Nuts, skins of fruits and berries, and whole grain foods (which contain the hulls of cereals) give larger residues. These characteristics depend on the fiber or hemicellulose

content (Table 59). The effects of polysaccharide residues on fecal bacteria have been summarized on page 154.

TABLE 59
FOODS WITH HIGH FIBER CONTENT¹
(Indigestible Residue)

	PER CENT
Bran	8.5
Guavas	5.5
Bran flakes	5.1
Blackberries	4.1
Brazil nuts	3.9
Cocoanut	3.4
Almonds, currants, lentils	3.0
Raspberries (red)	2.8
Shredded wheat biscuit	2.6
Grape nuts, peanuts	2.4
Parsnips, peas (green, shelled), pecans	2.2
Chocolate, walnuts	2.0
Dandelion greens, figs, onions	1.8
Popcorn, puffed wheat, raisins	1.7
Graham crackers, olives, peppers	1.5
Avocados, beans (string), cranberries, pears	1.4
Broccoli, pumpkin, strawberries, whole wheat bread	1.2
Blueberries, carrots, turnips	1.1
Apples, beets, cabbage, cauliflower, eggplant, okra, sweet potatoes, tangerines	1.0

¹ Per cent of fiber based on edible portion.

ABSORPTION

The monosaccharide digestion products are absorbed rapidly and completely by the normal small intestine. When small portions reach the large intestine abnormally, they can be absorbed slowly by the colon (about 1 gm. per hour in man); no sugar passes through the stomach wall. From the intestine, the absorbed sugars diffuse directly into the portal circulation and cause an increase in the blood sugar which parallels the rate of absorption. Since the blood sugar level is also affected by other factors, the best method for studying the rate of absorption is to determine the sugar which remains in the intestine (Cori's method). Sugars are absorbed from hypotonic, isotonic, and hypertonic solutions. The dead intestine, or an intestine cooled to 0° C., allows diffusion of dilute solutions of various soluble sugars at slow and approximately equal rates. Thirteen per cent sugar solutions diffuse equally and rapidly through living intestine.

After normal meals, the monosaccharide concentration in the lumen of the small intestine is maintained near 4 per cent, partly by dilution with digestive juices and partly by the gradual digestion of polysaccharides. At such intermediate concentrations, the small intestine exhibits a marked selectivity for sugars. *d*-Glucose and *d*-galactose are absorbed from three to four times as rapidly, and *d*-fructose, *d*-xyloketose, and *d*-xylose about twice as rapidly, as are *l*-arabinose, *d*-arabinose, *d*-mannose, *l*-sorbose, *l*-rhamnose, *d*-ribose, or *l*-xylose. *d*-Galacturonic acid is absorbed very slowly. Since monosaccharides are absorbed from the peritoneal cavity at equal rates, it is obvious that the intestine must have a special regulatory mechanism. The rapid intestinal absorption of glucose and galactose is inhibited by phlorhizin or iodoacetic acid poisoning. These substances, which either directly or indirectly block the phosphorylation of carbohydrates in cells, do not abolish the slow diffusion of sugars by osmotic forces. Phosphorylase and phosphatase activities of the intestinal epithelium are evidently responsible for selective sugar absorption. Glucose, galactose, and, to a lesser degree, fructose, are converted to phosphate esters in the mucosa during absorption; the esters are subsequently hydrolyzed by phosphatases and the liberated monosaccharides enter the portal blood. Fructose-phosphate ester is hydrolyzed rather slowly, and the fructose-1-phosphate concentration of the liver and intestinal mucosa increases after fructose feeding. The mucosa can convert a portion of fructose to glucose during absorption. Phosphorylation increases the diffusion gradient for glucose and galactose, and maintains their rate of absorption independent of ordinary changes in the intestinal concentration of these sugars. The diffusion of other sugars increases with the concentration. Starvation, thiamin deficiency, low carbohydrate diets, and deficiency of thyrotropic, thyroid, or adrenal cortical hormones decrease the rate of glucose absorption. Hyperthyroidism increases it.

Maltose and sucrose are removed from the intestine almost as rapidly as glucose, owing to the rapid hydrolysis of these disaccharides; cellobiose is well utilized, while lactose is hydrolyzed slowly, and it is absorbed even more slowly than fructose. Under normal conditions, the food disaccharides are completely hydrolyzed in the gastro-intestinal tract; but after the ingestion of large amounts some disaccharides (particularly lactose) are not entirely digested. Under these circumstances, the undigested sugar reaches the large intestine and stimulates bacterial fermentation; a portion of the sugar will diffuse through the intestine into the blood stream. Absorbed lactose and sucrose act as foreign substances. They are hydrolyzed with difficulty by general tissues, and are excreted by the kidney. The latter is the only internal organ which possesses appreciable quantities of β -glucosidase (lactase). Intravenously injected disaccharides or polysaccharides, other than maltose and glycogen, are also excreted. The intravenous injection of certain polysaccharides causes toxic disturbances; even intravenous glycogen causes leukopenia, followed by leuko-

cytosis, anemia, hepatic fatty infiltration, and lesions of blood vessels. Inulin may be safely injected as a test of kidney function. This soluble polysaccharide is excreted quantitatively by the normal kidney.

The capacity of intestinal carbohydrases to hydrolyze disaccharides may be measured by the appearance of the sugars in the urine. The smallest oral dose of any particular sugar which results in appreciable urinary sugar excretion is known as the *assimilation limit*. Average assimilation limits are as follows: glucose, fructose,¹ maltose, and sucrose, above 100 gm.; dextrins, 75 gm.; lactose, 50 gm.; galactose, 40 gm. for females and 30 gm. for males; and pentoses, 1 gm. Starch has no assimilation limit. In normal persons, it causes no urinary sugar excretion at any dietary level that the gastro-intestinal tract can accommodate.

BLOOD SUGAR

Absorbed monosaccharides are transported by the portal blood to the systemic circulation. Glucose readily penetrates human erythrocytes; it is distributed between plasma and erythrocytes in a ratio of 1.00 to 0.77, corresponding to the distribution of water. After a night's fast, normal venous blood contains 80 ± 20 mg. per cent of *d*-glucose and about 20 mg. per cent of "saccharoids" or non-glucose reducing substances, such as glutathione, thioneine, ascorbic acid, and the sugar of normal urine. The reducing value of saccharoids, in terms of glucose, is 40 mg. per cent in the erythrocytes and 8 mg. per cent in the plasma. These non-fermentable substances reduce the Folin-Wu, Shaffer-Hartmann, and Hagedorn-Jensen sugar reagents which, therefore, give normal blood sugar values of 100 mg. per cent. Since the blood saccharoid concentration is rather constant, the total reducing value of tungstic acid blood filtrates is clinically considered to be equivalent to blood glucose. When zinc salts are used to precipitate blood proteins, or when unclaked blood is employed, the saccharoids are largely eliminated from the filtrate. Benedict's alanine-copper reagent gives empirical values for tungstic acid filtrates that approach the true glucose content; however, the method is complicated by unequal fading of colors in the standard and the unknown. A more reliable procedure is to determine the true glucose by difference in reducing values before and after short period fermentation. Adult blood contains only traces of *d*-fructose, but appreciable quantities have been reported in embryonic blood and amniotic fluid.

In addition to reducing sugars, human blood contains about 10 mg. per cent of non-protein hydrolyzable sugar and a variable quantity (approximately 550 mg. per cent) of prosthetic polysaccharides attached to the plasma proteins. The serum of normal adults contains 95 ± 20 mg. per cent of glucosamine (in the prosthetic polysaccharides).

¹ About 10 per cent of normal individuals have a lower assimilation limit for fructose.

Carbohydrate Tolerance

After the average meal, the reducing sugar of blood increases from 20 to 40 mg. per cent above the normal fasting level. An elevation of the blood sugar level is termed *hyperglycemia*, while a decrease below the normal fasting level is termed *hypoglycemia*. The duration of the alimentary or post-prandial hyperglycemia varies with the quantity of glucose ingested. The maximal level attained in diabetic patients increases with the dosage, whereas in normal persons the dosage has little influence. The administration of fructose or starch causes less hyperglycemia than equivalent quantities of glucose or galactose.

The variations in the blood sugar following the administration of 1.5 gm. of glucose per kg. of body weight ¹ to resting patients (who have previously

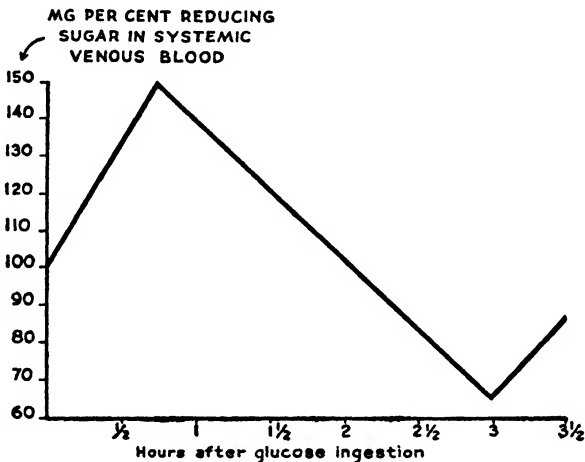


FIG. 6. Normal carbohydrate tolerance curve (1.5 gm. glucose/kg. of body weight *per os*).

received a normal diet for several days and have fasted for twelve hours) constitutes the clinical diagnostic *carbohydrate tolerance test*. The blood sugar is determined before, and at definite periods after, the administration of the glucose. A normal carbohydrate tolerance curve is shown in Figure 6. The initial rapid hyperglycemia is due to influx of glucose from the intestine. After forty-five minutes, the absorption of glucose is exceeded by its assimilation in the tissues, and the curve descends and reaches a subnormal level by the third hour. The slope of the descending portion of the curve is an index of the rate of glucose assimilation by the tissues, and is also a

¹ Infants require a greater, and aged persons a smaller, dosage per kg. of body weight to produce the same degree of hyperglycemia. Administration of 3 gm. glucose per kg. of body weight is suggested for infants.

useful test for diabetes. A quantitative method for evaluating the curve is provided by the *hyperglycemic index*, which is calculated as follows:

$$\frac{2 \text{ hour level} - \text{fasting level}}{\text{maximum level} - \text{fasting level}} \times 100$$

This index is zero for normal persons, but becomes increasingly positive with progressive delay in the utilization of absorbed glucose.

The fall in the blood sugar level is the result of storage and oxidation of carbohydrate, and the return of water from the intestinal tract to the tissues. The hypoglycemia is apparently caused by protracted operation of the hepatic storage mechanism which is stimulated by the hyperglycemia. The previous administration of carbohydrate normally decreases the hyperglycemia in a subsequent tolerance test ("Staub-Traugott effect"). Since this effect is not obtained in diabetics, it has been made the basis of a two dose clinical tolerance test in which one half the glucose is administered at the start of the test and the other half thirty minutes later.

Arterial blood tends to contain more glucose than does venous blood; the arteriovenous difference represents sugar assimilation by the tissues. In normal resting persons, this difference is only a few milligrams per cent but it increases after the injection of insulin or after the absorption of glucose or galactose (not fructose). At the peak of alimentary hyperglycemia, the arteriovenous difference averages 25 mg. per cent.

Control of Blood Sugar

Blood sugar levels are indices to glucose transport; they are largely determined by the balance existing between the hyperglycemia-provoking liberation of glucose from the liver, and the hypoglycemia-producing assimilation of this sugar by the general tissues. Stimulation of hepatic secretion of glucose is a characteristic effect of excess adrenaline, diabetogenic anterior pituitary extracts and thyroxine, and of insulin or thiamin deficiency. Hyperglycemia is produced by any process which causes sympathetic stimulation of the liver or of the adrenal glands, as, for example, asphyxia, exposure to cold, short strenuous physical effort, or severe emotional excitement. Injection of pilocarpine or corticosterone can also cause hyperglycemia. The intramuscular injection of 10 minims of 0.001 per cent adrenaline raises the blood sugar 40 mg. per cent within an hour, with a return to normal in two hours. Such injections have been used to test hepatic storage of carbohydrate.

Hypoglycemia results whenever glucose is assimilated faster by the general tissues than it is liberated from the liver. Hence, it can be produced by insulin in excess of the quantity which balances the anti-insulin or glycotropic factor of the anterior hypophysis and the hyperglycemia-producing hormones mentioned above. Hyperglycemia is also produced

by deficit of adrenal cortical hormone, severe depression of hepatic function, prolonged exercise and carbohydrate starvation, or by the injection of atropine or acetylcholine. However, clinical doses of atropine generally do not affect the blood sugar level. Children and physically active adults are especially susceptible to hypoglycemia during carbohydrate starvation. When the reducing sugar of the blood falls to a low level, *hypoglycemic shock* appears. The level at which shock occurs depends on the rate of fall of the blood sugar and on the sensitivity of the individual. In hypoglycemic shock, the residual reduction of the blood is largely due to saccharoids, and the true blood glucose is frequently near zero. The glucose deficiency stimulates certain brain areas which produce clonic convulsions; this is followed by paralysis of the central nervous system and cessation of respiration.

Immediate control of the blood sugar level resides in the liver; removal of this organ causes progressive depression of the blood sugar and death from hypoglycemia. When glucose is administered to hepatectomized animals, an exaggerated hyperglycemia appears. Also, when the important balancing hormones of the pancreas and the anterior pituitary lobe are absent, the blood sugar exhibits markedly abnormal fluctuations in response to starvation, adrenaline, or food ingestion. Liver function is regulated by two antagonistic classes of hormones: insulin, which favors storage, *versus* adrenaline, diabetogenic factor and thyroxine, which cause hepatic secretion of glucose. Insulin is also directly antagonized, even in hepatectomized animals, by glycotropic extracts of the anterior lobe of the pituitary, which do not cause hyperglycemia *per se*. Corticosterone assists hepatic storage of glycogen in adrenalectomized animals; but it counteracts insulin hypoglycemia in hypophysectomized animals (pages 342 and 702. Another adrenal cortical hormone, compound E (dehydrohydroxycorticosterone), can induce hyperglycemia. This elaborate hormonal regulation of carbohydrate metabolism is established at the mid-point of embryonic life, when the islands of Langerhans begin to function. At earlier stages, the embryonic blood sugar is maintained by placental regulation and by influx of glucose from the maternal blood. The glycogen of leukocytes in tissue culture is insensitive to insulin, thyroxine, or adrenaline.

TISSUE SUGARS

Glucose and other monosaccharides diffuse freely through capillaries; the average quantities of reducing sugar in normal tissues and tissue fluids are recorded in Table 60. Sugars which are not utilized are retained only temporarily by tissues, whereas glucose is assimilated rapidly by cells and used as a preferred source of energy. The concentration of reducing sugar in the liver, lymph, and transudates approximates that of blood. The glucose content of these tissues, of the kidney, and of cerebrospinal fluid fluctuates with the blood sugar level, while muscle, skin, and brain

TABLE 60

TISSUE CARBOHYDRATES

(Mg. Per Cent, as Glucose)

TISSUE	TOTAL REDUCING SUGAR	FERMENTABLE SUGAR	GLYCOGEN
Blood	100 \pm 25 ¹	80 \pm 20	Trace
Lymph, transudates	100	80	Trace
Liver	100		5,000 (200-15,000)
Skin :	70	55	75
Brain		60	100
Urine	60	12	None
Kidney	60		150
Cerebrospinal fluid	55 \pm 15 ²	45	Trace
Muscle	30	12	1,000 \pm 800
Heart		35	500
Lung	30		
Intestine	25		
Saliva and gastro-intestinal secre- tions	25	Traces	

¹ In the newborn, 70 \pm 15 mg. per cent.² In children, 80 \pm 10 mg. per cent.

glucose is more constant. In fact, the reducing sugar of brain is more easily decreased by the administration of strychnine than by hypoglycemia.

Blood plasma contains only traces of glycogen; but this carbohydrate and the prosthetic polysaccharides of proteins are characteristic constituents of the cells of adult animals. Glucose must be continually supplied to mammalian cells, and in the cells it must be polymerized to glycogen in order to prevent degenerative changes. Glycogen increases in cardiac muscle, and in the cortical cells of the brain, as a reaction to injury. Prior to the establishment of the insulin mechanism, glycogen plays a less significant role in the carbohydrate metabolism of the embryo. Ovomucoid prosthetic polysaccharide, which contains acetylglucosamine, galactose, and mannose units, is a source of energy in the early embryonic life of the chick. Primitive connective tissue, bone, and other tissues require a glycoprotein matrix for normal cell proliferation. This matrix is present in Wharton's jelly of the umbilical cord; in adult tissues it is overshadowed by cells, except in the vitreous humor of the eye.

✓ GLYCOGENESIS AND GLUCONEOGENESIS

1 The transformation of glucose to glycogen is termed *glycogenesis*. In a normal adult man, there are approximately 300 gm. of muscle glycogen and from 150 to 200 gm. of liver glycogen. All tissues can form glycogen

from glucose; but the liver is a special glycogenic organ, and glycogen is stored in greater concentrations in the liver than in other tissues. The liver also differs from the general tissues in its ability to reform free glucose from the stored glycogen, and to secrete glucose into the blood for transport to other tissues. The formation of glucose from glycogen is a function of a specific hepatic glucophosphatase. Experiments with deuterium oxide in rats show that hepatic glycogen has a half life of 1 day, as compared with 3 to 4 days for glycogen elsewhere in the body./

A third and unique hepatic function is *gluconeogenesis*, or formation of glucose and glycogen from certain non-glucose substances. Hepatic gluconeogenesis is stimulated by lack of insulin, also by excess of thyroxine, diabetogenic factor, corticosterone, hydroxycorticosterone and compound E, and by the administration of phlorhizin. The liver can produce glycogen from fructose or lactic acid twice as rapidly as from glucose, dihydroxyacetone, or glycerol, and four times as rapidly as from galactose or mannose. Alanine, glycerose, and pyruvic acid are transformed to glycogen very slowly. Other gluconeogenic substances include maltose, mannose, *l*-sorbose, *d*-sorbitol, *l*-xyloketose, and certain amino acids and acids derived from carbohydrates (Table 40, page 226). During prolonged fasting, hepatic gluconeogenesis from proteins, lactic acid, and glycerol prevents fatal hypoglycemia. It has been claimed that acute inflammation incites abnormal local gluconeogenesis in non-hepatic tissues.

(Fructose, galactose, and dihydroxyacetone are changed to glycogen only in the liver and in the intestinal mucosa; insulin is not necessary for these particular transformations. Tolerance to fructose has, therefore, been suggested as a test of liver function. Only slight hyperglycemia results from the ingestion of fructose, because of slow absorption and rapid hepatic glycogenesis. The greater part of circulating fructose and galactose is removed from the blood by the liver.

Sugars which do not form glycogen are not significant sources of energy in animals, although they are utilized by micro-organisms. Hence, glycogenesis not only serves as a storage mechanism but, in most animal tissues, it is also a preliminary reaction to the oxidation of carbohydrate.⁴ Glycogenesis is dependent on phosphorylation and aerobic oxidation; it does not occur anaerobically, and amylases are not concerned. In tissues, glucose is phosphorylated before being polymerized; the glycogen phosphorylase of animal tissues is an adenylic acid-protein complex whose activity is accelerated by magnesium cations and inhibited by phlorhizin, glucose, or β -glycerophosphate. Magnesium and potassium ions, in the concentrations found in intracellular fluid, accelerate glycogen synthesis by liver slices. Skeletal muscle contains about 60 mg. per cent of polysaccharide phosphorylase. The formation of glucose-1-phosphate requires transfer of energy from carbohydrate catabolism through energy-rich phosphate (adenosine triphosphate), while the subsequent polymerization to polysaccharide does not. | The polymerization is catalyzed or primed by

the presence of glycogen or starch, since glucose-1-phosphate reacts with the terminal units of the polysaccharide chain or its branches. Glycogen and starch have been synthesized *in vitro* from glucose-1-phosphate by the action of phosphorylases prepared from liver, heart, brain, and yeast, and from muscle and potatoes, respectively. Phosphorylases synthesize sucrose in plants, while bacteria produce levans by glycoside linkage exchange.

The major portion of a carbohydrate meal is distributed to the general tissues, where it undergoes glycolysis and oxidation; about 25 per cent is stored as hepatic glycogen. Carbohydrate in excess of the requirements of the general tissues and the storage capacity of the liver is converted to fat (page 228).

Glycogenic Factors

Substances which are known to stimulate glycogenesis include: insulin, corticosterone, the glycostatic factor of the anterior pituitary gland, and thiamin. The anterior lobe of the pituitary is intricately concerned, for it secretes a corticotrophic hormone which stimulates the production of corticosterone. Insulin is a most significant and powerful accelerator of glycogenesis. Hyperglycemia stimulates insulin secretion and, therefore, glycogenesis. In diabetic animals, glycogenesis is inhibited because of insulin deficit and only small temporary deposits of liver glycogen appear in response to alimentary hyperglycemia. Stimulation of glycogenesis in the liver and other tissues is the most important effect of insulin. In man, insulin acts for from two to six hours following subcutaneous injection. Some enzymatic or sulfhydryl system of the blood and tissues gradually inactivates the insulin. As the body temperature is lowered in cold-blooded animals, the latent period of the insulin effect is prolonged from one half to six days.

Removal of the adrenal glands lowers liver glycogen, while removal of the pituitary gland stabilizes it; such animals exhibit hypoglycemia, and are very sensitive to insulin. The glycotrophic factor of the anterior hypophysis has a specific anti-insulin action, even in hepatectomized animals. Its activity is mediated partly by corticosterone, hydroxycorticosterone, and compound E of the adrenal cortex. (See page 690.) An inhibition to the glycogenic function of insulin develops during prolonged hypoglycemia in fasting animals. This condition is not primarily due to an excess of adrenaline or to a deficit of insulin. It is not affected by adrenalectomy or evisceration; but the ingestion of a single carbohydrate meal abolishes the insulin-inhibition. The phenomenon resembles the Staub-Traugott effect (page 304). Prior to the formation of the islands of Langerhans, injected insulin does not lower the blood sugar of embryos.

Insulin is a protein formed in the β -cells of the islands of Langerhans. The chemistry of this hormone is considered in Chapter VI. Insulin is inactivated by sulfhydryl compounds and by other reducing and oxidizing

agents; its physiological activity depends on the integrity of its —S—S— or cystine radicals. Crystalline insulin and zinc insulin have biological activities equivalent to approximately 21 and 25 international units per mg., respectively. Normal human blood contains about 0.02 unit of insulin per 100 ml. The secretion of insulin and the insulin content of the pancreas are decreased by starvation, by high fat diets, and by insulin administration. Repeated injections of increasing doses of crude anterior pituitary extracts cause degeneration of the islands of Langerhans and a decreased insulin output in dogs, cats, and rabbits, but not in rats, mice, or guinea pigs. The interrelations of the pituitary gland and pancreas are thus somewhat obscure, but there is little doubt that insulin production is not directly dependent on other hormones, and that insulin secretion is stimulated by hyperglycemia and depressed by hypoglycemia. The vagus innervation and the pituitary gland are not essential for these effects. Secreted insulin arrives first at the liver. When extra insulin is administered to normal animals it stimulates glycogenesis chiefly in the general tissues, since the liver is already well supplied with the hormone. Insulin causes active transfer of glucose from body fluids into cells, and hence it stimulates glycogenesis and hypoglycemia in hepatectomized animals. It not only increases the liver glycogen, but it also redistributes carbohydrate in the direction of the general tissues.

The administration of insulin to normal animals augments cardiac glycogen. The latter also increases during starvation, and in diabetes mellitus when the glycogen of the liver and of the skeletal muscles is decreasing. Brain contains little glycogen; it is highly dependent upon a continuous supply of glucose, as evidenced by the occurrence of hypoglycemic shock. The glycogen content of the brain is apparently uninfluenced by hyperglycemia, fasting, or pancreatectomy; but insulin overdosage sufficient to cause hypoglycemic convulsions has been reported to decrease brain glycogen by 50 per cent. Progesterone, the hormone of the corpus luteum, stimulates glycogen formation in uterine epithelium. The glycogen content of the endometrium is approximately 170 mg. per cent at the proliferative phase, and 1100 mg. per cent at the early differentiative phase (eighteenth day of the menstrual cycle). Estrogens are important for the mobilization of glycogen in the vaginal epithelium, where the polysaccharide increases during the cycle and falls in the late premenstrual phase. The gynecologist stains the cervix with compound solution of iodine (Lugol's solution) to detect cancer tissue, since it contains much less glycogen than the neighboring normal tissue.

GLYCOGENOLYSIS AND GLYCOLYSIS

The degradation of glycogen to glucose is termed *glycogenolysis*; this reaction was long attributed to the traces of amylases present in tissues and tissue fluids. Hepatic glycogenolysis produces glucose, but not dextrin

or maltose; it is a result of phosphorolysis by phosphorylase and of hydrolysis by a glucophosphatase found only in the liver (Fig. 7, page 316). The disappearance of glycogen from tissues, other than the liver, is not accompanied by the liberation of glucose. Lactic acid is the characteristic stable product of anaerobic catabolism of glucose and glycogen in the general tissues. *Glycolysis*, or the production of lactic acid, requires phosphorylation reactions which are inhibited indirectly by iodoacetic acid, but not by insulin deficiency. Thiamin deficit stimulates glycolysis, particularly in the brain and kidney. Phlorhizin and adrenaline accelerate glycolysis in muscles, but not in the brain or in the heart; the action of these substances is not inhibited by insulin or ergotoxine.

Liver cells do not contain the active glycolytic enzyme system which causes the production of lactic acid in the general tissues. Lactic acid escapes in small quantities from the tissues into the blood. Most of the lactic acid is transported to the liver which converts it to glycogen, and subsequently restores it to the blood stream and tissues as glucose. The liver is, therefore, the direct source of all endogenous blood glucose, although muscle glycogen can indirectly replace blood sugar, via the lactic acid cycle. Thus, fasting animals can increase their liver glycogen, at the expense of muscle glycogen, during long strenuous exercise when considerable quantities of lactic acid are liberated from the muscles. The brain, heart, and general musculature also remove lactic acid from blood, but they do not reliberate it as glucose. Cardiac and skeletal muscles can either convert lactic acid to glycogen, or oxidize it; the brain can only oxidize it.

During contraction, the glycogen of muscle cells is glycolyzed rapidly; four fifths, or more, of the lactic acid thus formed is resynthesized to glycogen during the subsequent muscular relaxation. This synthesis does not require insulin, in contrast to the replacement of oxidized glycogen from blood glucose. Diabetic animals replace their oxidized muscle glycogen very slowly; starving animals also require about twelve hours for restitution. The glycostatic factor of the anterior hypophysis aids in the maintenance of muscle glycogen. Since thiamin definitely accelerates the synthesis of glycogen from lactic acid, oxidative decarboxylation may be involved in this process (page 324).

Hepatic glycogenolysis, like tissue glycolysis, is stimulated by asphyxia; it is also accelerated by acidosis, exercise, starvation, administration of phlorhizin, thiamin deficit, exposure to cold, and sympathetic stimulation of the liver. The hormones which accelerate glycogenolysis in the liver include: adrenaline, the diabetogenic factor, and thyroxine. Bernard's piqure, or puncture of the floor of the fourth ventricle, causes sympathetic stimulation, adrenaline discharge, and rapid intensive hepatic glycogenolysis. Hypoglycemia stimulates adrenaline secretion; and it is, therefore, a part of the mechanism for maintaining the blood sugar level. The glycogenolytic action of adrenaline is decreased by hypophysectomy, and

TABLE 61
ROLE OF HORMONES AND VITAMINS IN
CARBOHYDRATE METABOLISM

HORMONE	SECRETION		PRINCIPAL EFFECTS
	Gland	Stimulated by	
Adrenaline	Adrenal medulla	Sympathetic nerves, asphyxia, hypoglycemia, thyroglobulin, pituitrin	Glycogenolysis, ¹ hyperglycemia, glycosuria, glycolysis (muscle)
Corticosterone ²	Adrenal cortex	Corticotrophic hormone of anterior pituitary	Gluconeogenesis, glycogenesis, hyperglycemia
Insulin	Pancreas	Hyperglycemia	Glycogenesis, hypoglycemia, decreases gluconeogenesis
Pituitary factors			
Diabetogenic factor ³	Anterior pituitary		Glycogenolysis, hyperglycemia, glycosuria, gluconeogenesis
Glycostatic factor	Anterior pituitary		Glycogenesis (muscle)
Glycotrophic factor ³	Anterior pituitary		Prevents insulin hypoglycemia
Thyroglobulin	Thyroid	Thyrotrophic hormone of anterior pituitary	Glycogenolysis, hyperglycemia, gluconeogenesis

VITAMIN	ACTIVE FORM IN TISSUES	PRINCIPAL COENZYMIC EFFECTS
Nicotinic acid	Cozymase	Oxidation of glucose and its intermediates
Pantothenic acid		Oxidation of lactic and pyruvic acids
Riboflavin	Yellow enzyme	Oxidation of glucose and its intermediates
Thiamin	Coccarboxylase	Glycogenesis, oxidation of lactic acid and pyruvic acid

¹ In late phases of its activity, adrenaline leads to increased hepatic glycogenesis from lactic acid.

² Corticosterone and the related adrenal cortical hormones which have an oxygen atom at carbon 11 (compound E, dehydrocorticosterone, hydroxycorticosterone) are much more active in carbohydrate metabolism than desoxycorticosterone which affects mineral metabolism.

³ As explained on page 690, these pituitary activities are mediated to some extent by the corticotrophic hormone of the anterior pituitary gland which stimulates production of corticosterone and related hormones.

it is suppressed by ergotoxine and by adrenalectomy. Thyroxine causes slow, prolonged glycogenolysis; it probably operates through adrenaline or sympathetic stimulation. The diabetogenic factor is necessary for normal rapid glycogenolysis in the liver. Increased potassium in the blood and tissues, which occurs in adrenalectomized animals, inhibits hepatic glycogenesis. The complex effects of hormones, and of the vitamins, are summarized in Table 61. The metabolic effects of anterior pituitary extracts are summarized on page 689.

RÉSUMÉ

Liver glycogen maintains blood sugar levels and assists in the regulation of hepatic function, while the glycogen of general tissues serves as a local

fuel supply which is normally replenished by glucose liberated from the liver, or absorbed from the intestine. Glycogen storage is very important, since the free glucose of the body (about 20 gm.) could be exhausted by a quarter hour of vigorous exercise. During prolonged muscular activity there is active transformation of liver glycogen to muscle glycogen, via blood glucose. Two antagonistic groups of hormones constitute a regulatory superstructure which facilitates rapid mobilization of liver glycogen. In their absence, as in depancreatized-hypophysectomized animals, liver glycogen is less sensitively balanced at a low level. The placenta serves as a "temporary liver" for the embryo; it performs hepatic glycogenic functions during the first half of embryonic life (*i.e.*, until the liver assumes its adult characteristics). Then the hormone mechanisms are established and glycogen suddenly shifts from the placenta to the liver; it also increases in the general tissues of the embryo. Placental glycogen is not affected by adrenaline, insulin, or thyroxine; neoplasms and the tissues of newborn animals require enormous doses of adrenaline to shift their glycogen.

SUBSTRATES OF CARBOHYDRATE OXIDATION

Carbohydrate catabolism is the most fundamental energy-yielding process of living cells. Carbohydrates are more easily dehydrogenated than are either fats or amino acids, and they are the preferred biological sources of energy. Available carbohydrate causes unique sparing of other foods; especially important is the *protein-sparing action*, which lasts for several hours after carbohydrate ingestion. It represents decreased hepatic gluconeogenesis, and delayed oxidation of amino acids. The protein-sparing action of sugar renders it particularly valuable in the nourishment of patients who are severely ill. Intravenous injections of 5 or 10 per cent glucose solutions are commonly administered to patients who cannot ingest food. In diabetes mellitus, glycogenesis is decreased; carbohydrate is diverted from the general tissues and no longer exerts its protein-sparing action.

The energy from carbohydrate catabolism is necessary for numerous physiological functions, including such biological synthetic reactions as the formation of fat and glycogen from sugars, and the synthesis of acetylcholine, protein, and urea. It has been found that 5 to 10 per cent of a given quantity of administered glucose is oxidized during the glycogenesis of the remainder. The brain and retina use energy from carbohydrate catabolism almost exclusively. Peripheral nerves consume much less oxygen than does the brain; the respiratory quotient of resting nerves is low, but it rises during activity. Muscles can utilize carbohydrate, fat, or protein as sources of energy for contraction; however, carbohydrate is employed by this tissue under ordinary circumstances and for sudden muscular efforts. When fat oxidation is the source of energy, the muscular work is performed less economically, and fatigue sets in as the muscle glycogen becomes exhausted.

The principal *carbohydrate substrates* of cellular oxidizing enzymes are glycogen and glucose; other utilizable sugars, such as fructose, mannose, galactose, dihydroxyacetone, sorbose, mannoheptulose, and various sugar acids and alcohols, are largely converted to hepatic glycogen within two hours after administration to animals. Fructose and mannose can be oxidized directly, although more slowly than glucose or glycogen. Muscles and the liver oxidize glycogen preferentially, whereas, this polysaccharide is relatively inert in cancer tissue, the tissues of patients with von Gierke's glycogen disease, and in normal brain, retina, and embryonic tissue, which oxidize glucose, fructose, and mannose readily. The remaining utilizable sugars are oxidized slowly; only very small quantities of galactose, glucosamine, acetylglucosamine, ribose, xylose, or starch are utilized directly.¹ Brain, heart, and malignant tissues can utilize lactic and pyruvic acids, and many tissues oxidize slowly succinic, citric, and other acids derived from carbohydrates. Ethyl alcohol is not an important food, although small concentrations can be oxidized by animals; larger amounts are narcotic, and they inhibit carbohydrate oxidation. Insulin accelerates the hepatic oxidation of ethyl alcohol, and administration of pyruvic acid has a similar effect.

There are four important phases of carbohydrate catabolism: (1) phosphorylation, (2) glycolysis, (3) oxidation, and (4) fermentation. The latter is of interest only in the metabolism of micro-organisms.

GLYCOLYSIS

This process may be defined as the biological formation of two mols of *L*-lactic acid from one mol of hexose, or its glycogen equivalent. Glycolysis and alcoholic fermentation are anaerobic dismutations, or oxidation-reduction reactions. Glycolysis is not an essential prerequisite to carbohydrate oxidation, since iodoacetate-poisoned muscles contract and produce energy without the formation of lactic acid. Concentrations of iodoacetate which inhibit anaerobic glycolysis do not impair carbohydrate oxidation in the brain or in muscle. In general, very little lactic acid is formed during the aerobic oxidation of carbohydrate. Normal muscles can do work equivalent to two thirds of their maximal oxygen consumption without significant lactic acid accumulation. Nicotine abolishes cerebral oxidation of lactic acid but not that of glucose. While glycolysis and oxidation are regarded as separate anaerobic and aerobic phases of carbohydrate catabolism, the two processes are interrelated through common initial phosphorylation reactions (Fig. 7). Aerobic processes are essential for the assimilation of lactic acid from the blood and its utilization by the brain. Under anaerobic conditions, lactic acid diffuses from the tissues

¹ It is interesting that the administration of diets which contain 35 per cent galactose or xylose causes the development of cataract in rats. The mechanism of this action remains unexplained.

into the blood. The fact that carbohydrate can be utilized either aerobically or anaerobically renders it an efficient food for all cells. Anaerobic glycolysis releases only 32.6 Cal., as contrasted with 675 to 680 Cal. for the complete aerobic oxidation of 1 mol of glucose, or its glycogen equivalent. Nevertheless, glycolysis constitutes a valuable emergency or reserve mechanism of cells, since it requires no oxygen, and the energy of glycolysis can be used for the synthesis of energy-rich phosphates. Glycogen is a substrate for glycolytic enzyme systems; hence, glycogen-rich cells survive longest, anaerobically. Most tissues are less subject to degenerative changes if they are well supplied with glycogen.

The value of glycolysis in emergencies is illustrated by the events which follow muscular contraction. Lactic acid is liberated in the anaerobic phase of muscle metabolism, chiefly during relaxation, and 80 per cent or more is then rebuilt to glycogen during subsequent aerobic oxidation. At the start of exercise the muscle operates anaerobically, but after adjustments are made to provide adequate oxygen, lactic acid is no longer liberated. Any treatment which increases the lactic acid content of normal muscle also accelerates its oxygen uptake, and the resynthesis of glycogen. Thus, an aerobic resting muscle preparation in a solution of lactate or pyruvate oxidizes about 20 per cent of the substrate and synthesizes carbohydrate from the remainder. The increased oxidation in an active muscle is not due to acidity changes; the muscle maintains its pH except when excessive amounts of lactic acid accumulate, as in fatigue. Hydrogen ions tend to repress both the oxidation and the glycolysis of carbohydrate. As contrasted with the resting metabolism of muscle, its increased oxygen consumption during activity is inhibited specifically by azide and is evidently due to the activity of cytochrome oxidase or of a copper-containing enzyme. We are thus led to the conclusion that aerobic oxidation is relatively independent of the stabilization products of glycolysis (lactic, pyruvic, triose monophosphoric, and hexose diphosphoric acids). Pyruvic acid, sometimes called coferment T, stimulates maximal glycolysis in the brain, embryonic tissue, and cancer tissue, which glycolyze glucose in preference to glycogen.

Glycolysis makes possible short periods of extra work during the partial asphyxia of strenuous exercise; it results in marked lactic acid accumulation in the muscles and in the blood. In this way an *oxygen debt* is established, and a long recovery period is necessitated. By severe exertion, a man can establish a debt of 10 to 30 liters of oxygen requiring more than 45 minutes for correction. During moderate exercise increased aerobic oxidation in muscles maintains the blood lactic acid at a comparatively low level. This adjustment is known as the *steady state*. The heart can function for hours in the absence of oxygen, provided its perfusion fluid is kept slightly alkaline, as for example, by incorporating sodium lactate into the perfusion fluid; under these conditions it operates by means of

glycolytic energy. Cardiac failure develops only when the heart glycogen is completely glycolyzed; the organ may then be revived by providing it with glucose. Iodoacetate stops anaerobic glycolysis in the heart, but does not affect its aerobic operation.

These illustrations indicate that lactic acid is a product of carbohydrate metabolism, when oxygen is deficient and a portion of the carbohydrate functions as hydrogen acceptor. Aerobic oxidation is necessary to reverse glycolysis completely.

Normal brain and resting muscle contain approximately 20 mg. per cent lactic acid, as compared with the fasting blood level of 15 mg. per cent (page 27). Normally, lactic acid cannot accumulate in the liver because of rapid transformation to glycogen. Glycolysis is most rapid in the brain, retina, embryonic and cancer tissues; intermediate in muscle, kidney, intestinal mucosa, cartilage, and connective tissue; and very slow in many glands, including the liver, pancreas, salivary glands, and testicle. The metabolism of spermatozoa is primarily glycolytic. The rate of glycolysis in embryonic and cancer tissues is about eight times that in muscle working at its maximum. The ability of embryonic cells to grow anaerobically decreases during development. Anaerobic glycolysis of embryonic tissue is inhibited by *l*-glycerose. Most adult tissues have a characteristic low glycolytic rate. The exceptions are the retina, gray matter of the brain, and chorionic epithelium of the placenta, all of which exhibit embryonic characteristics. Glycolysis in retina, brain, and muscle can be inhibited by glutamic acid, which allows the conversion of pyruvic acid to alanine by transamination.

Glycolysis does not occur to any extent in normal cells adequately supplied with oxygen. The repression of glycolysis in normal tissues by oxygen is termed the *Pasteur effect*. (See page 90.) Potassium and sodium cations, when added to brain slices *in vitro*, stimulate carbohydrate oxidation and depress anaerobic glycolysis. Unlike normal tissue, leukemic leukocytes and cancer cells do not show any Pasteur effect. Malignant tumors may, therefore, be regarded as anaerobic parasites which compete with and encroach on normal tissues by a superior utilization of glycolytic energy for life and growth. In this respect, as well as in their disorganization, the neoplastic tissues resemble colonies of anaerobic micro-organisms. When the enzyme systems of embryonic tissues are poisoned by small quantities of cyanide or arsenic trioxide, the transplanted cells can become malignant. The high glycolytic rates found in chorionic epithelium and in retina may be related to the short induction periods of malignant epitheliomas of these tissues, and to the invasive properties of fetal trophoblasts. Glycolysis by erythrocytes and leukocytes, *in vitro*, causes the disappearance of glucose from normal blood specimens in six hours, at 37° C. Glucose disappears even more rapidly from the blood of patients with polycythemia or chronic myelogenous leukemia. Reticulo-

cytes exhibit more rapid glycolysis and aerobic metabolism than the mature erythrocytes. When the analysis of blood must be delayed, the chemist prevents glycolysis by adding sodium fluoride (Fig. 7).

AEROBIC OXIDATION

Normally, the oxidation of carbohydrate provides approximately two thirds of the human energy requirement. Before considering the details of aerobic oxidation, it is desirable to clarify the significance of respiratory quotients. The complete combustion of carbohydrate to carbon dioxide and water gives a R.Q. (respiratory quotient) of 1.0, which is higher than the usual quotients observed for the general metabolism of most tissues. It has frequently been assumed that an increase in the R.Q. towards 1.0 invariably indicates enhanced oxidation of carbohydrate. Insufficient attention has been paid to the increases due to glycolysis, and to the formation of acid metabolites which liberate carbon dioxide from bicarbonate. Also, gluconeogenesis from protein or fat, which is so prominent in starvation, diabetes, and phlorhizin poisoning, causes a considerable lowering of the R.Q. without necessarily hindering carbohydrate oxidation. Variations of R.Q. are, therefore, seldom admissible as critical indices of the rate of carbohydrate combustion. The ingestion of sucrose, fructose, dihydroxyacetone, or galactose causes a higher and more rapid rise in the R.Q. of animals than the administration of equivalent quantities of glucose; but, with the exception of galactose, these sugars also stimulate glycolysis and cause lactic acidemia with a resultant false carbon dioxide output. Glucose administration, muscular exercise, and injections of adrenaline raise the R.Q. of a normal animal, but not the low respiratory quotient of a diabetic animal.

Diabetes mellitus is a type of carbohydrate starvation resulting from disturbed glycogenesis and gluconeogenesis; the basal metabolic rates of these patients are as low as those of normal fasting persons (page 124). Insulin represses gluconeogenesis and glycogenolysis in diabetics, and restores the normal effect of glucose ingestion on the respiratory quotient; but it does not directly affect the enzymatic oxidation of glucose, fructose, or lactic acid in cells. That insulin is unnecessary for biological carbohydrate oxidation has been proved by studies of depancreatized hepatectomized dogs. Removal of the liver of a diabetic animal raises the R.Q., just as the administration of insulin does. During hypoglycemic shock from excess insulin, cerebral oxidation is depressed because of deficient transport of glucose to the brain. Insulin is necessary to assure optimal tissue glycogenesis and normal hepatic function, and to prevent the loss of glucose in the urine. This hormone diverts glucose to the tissues, where the sugar is converted to glycogen and oxidized by enzyme systems. It has frequently been stated that the daily administration of 1 unit of insulin (45 γ of the crystalline hormone) to a diabetic will prevent the

urinary loss of 2 gm. of glucose. Because of the indirect action of insulin on carbohydrate combustion, the glucose equivalent of 1 unit of the hormone actually varies from 1 to 4 gm., depending on the severity of the disease and the sensitivity of the patient to insulin.

Thiamin deficit decreases the R.Q., lowers the oxygen uptake, and inhibits oxidation of lactic acid in the brain. The coenzymic role of this vitamin in carbohydrate metabolism is discussed later (page 324). Anterior pituitary extracts have little influence on carbohydrate oxidation in hepatectomized dogs. Narcotics and mescaline tend to depress oxygen consumption and to inhibit the oxidation of glucose, lactic acid, and pyruvic acid in the brain; amines derived from leucine, tyrosine, and tryptophane produce similar effects. By decreasing the available energy, narcotics depress the normal functional activity of the central nervous system in a reversible manner. In the case of morphine, the depression becomes measurably less during habituation to the drug. Adrenaline and thyroxine raise the metabolic rate and accelerate carbohydrate oxidation in the tissues. The increased R.Q. which is produced by adrenaline is traceable to accelerated glycolysis and ketogenesis. Iodoacetate-poisoned muscles have a low R.Q., which can be raised by perfusion with lactate.

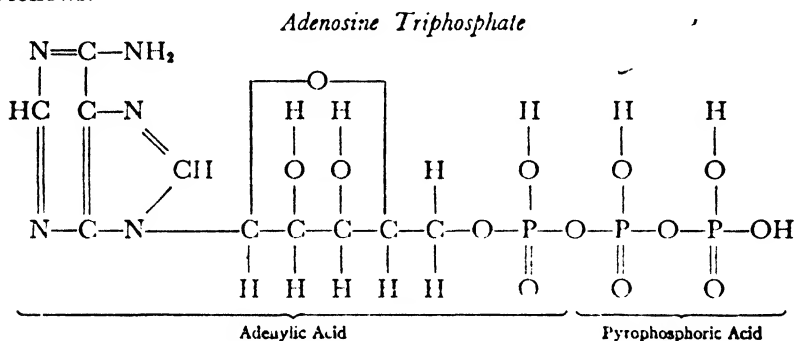
PHOSPHORYLATION AND TRANSPHOSPHORYLATION

There is an important mechanism in cells which converts oxidation-reduction energy (particularly energy from the oxidation of carbohydrates, α -keto acids, and dicarboxylic acids) into phosphate bond energy. The efficiency of conversion of the energy from carbohydrate oxidation to utilizable energy of phosphate bonds is approximately 60 per cent. The group potential of ordinary phosphate esters is only 3 Cal., but there is a series of phospho compounds which have energy-rich linkages with a group potential of 10 to 11 Cal. Included in the latter category are acetyl phosphate, adenosine triphosphate, phosphoarginine, phosphocreatine, and phosphopyruvic acid, which can transfer their phosphate radicals to other compounds and thus perform a variety of transphosphorylations. The biological formation of such energy-rich phosphates is intimately linked with dehydrogenation reactions.

In general, phosphorylation of carbohydrate in tissues precedes glycolysis, oxidation, and alcoholic fermentation (Fig. 7). Esterification with phosphoric acid provides sugars with a prosthetic radical which can readily unite with enzymatic proteins. The phosphorylation of sugars in the tissues is accelerated by phosphorylases (Fig. 7). In normal muscle, glycogen is the principal substrate of the phosphorylases; glycolysis in muscles ceases when phosphate is unavailable. Thus, phosphorylation is necessary for the catabolism of glycogen, glycolysis, and a number of types of fermentation. However, phosphorylation is not a necessary prerequisite for all types of carbohydrate oxidation. In brain, retina, embryonic

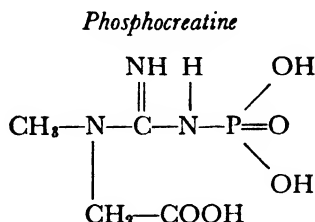
tissue, and cancer tissue, glucose and fructose can be dehydrogenated without phosphorylation, if bicarbonate is present. Also, the liver and certain fungi and bacteria can metabolize carbohydrate by means of glucose dehydrogenase without phosphorylation. (See upper right portion of Fig. 7.)

The chief coenzyme for phosphorylation is a phosphate donor, *adenosine triphosphate* (adenyl pyrophosphate), although it is claimed that cozymases have a similar function, under certain conditions, and adenylic acid is the cozymic radical for glycogen phosphorylase. Small quantities of adenosine triphosphate are present in all living cells; muscle, brain, and yeast contain comparatively large amounts, and human erythrocytes have approximately 95 mg. per cent. The formula for adenosine triphosphate is as follows:

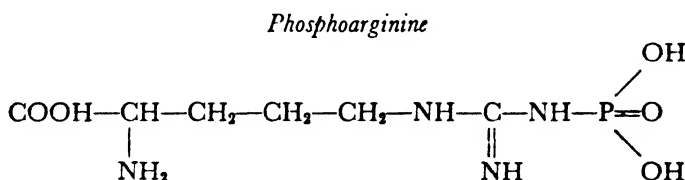


Two of the phosphate radicals of adenosine triphosphate are readily interchanged with inorganic phosphate, as shown by studies with phosphorus isotope. Adenosine triphosphatase, which removes one phosphate radical, is found in the heart, liver, kidney, and muscle. Myosin, the contractile protein of muscle, is a very active adenosine triphosphatase, activated by calcium ions. The phosphate liberated from adenosine triphosphate can be transferred directly during transphosphorylation of glucose, fructose, hexosemonophosphate, and trioses, as proved by experiments with radioactive phosphorus (Fig. 7). The transferred phosphate eventually returns from glycolytic intermediates (phosphopyruvic acid and acetyl phosphate) to unite directly with adenylic acid and adenosine diphosphate to resynthesize adenosine triphosphate. The resynthesis is accelerated by potassium ions and inhibited by calcium ions. When the return of phosphate from this source is prevented, as in muscles poisoned with fluoride or iodoacetate, the adenylic acid is deaminated, by a cellular deaminase, to ammonia and inosinic acid (page 518). In iodoacetate-poisoned muscle, the adenosine triphosphate continues to phosphorylate hexose. Insulin increases the turnover of adenosine triphosphate in liver and muscles in association with the increased assimilation of glucose. Phlorhizin tends to inhibit phosphorylases, and fluoride is inhibitory to the phosphatases.

The labile substance, *phosphocreatine* (creatine phosphoric acid, or phosphagen), serves as a reservoir of phosphate which is enzymatically transferred to adenylic acid when other phosphate transfers are not operating at sufficient velocity. In Figure 7, note that the phosphocreatine-adenosine triphosphate cycle is thermoneutral. The formula for phosphocreatine is as follows:



This phosphagen is found in striated muscle and in nervous tissue of vertebrates; it has a much more limited distribution in tissues than does adenosine triphosphate. In invertebrate tissue, it is replaced by another phosphagen, namely, phosphoarginine:



The hydrolysis of phosphocreatine is related to muscle excitation, as measured by chronaxie. Curarine, fatigue, and degeneration of motor nerves inhibit the phosphocreatine breakdown in muscle, while veratrine has an opposite effect. Strychnine decreases the chronaxie of nerves, but does not affect the hydrolysis of phosphocreatine in muscles. These substances do not influence the resynthesis of phosphocreatine. In muscles poisoned with small quantities of iodoacetate, phosphocreatine continues to be hydrolyzed but it is no longer resynthesized anaerobically. However, when oxygen is admitted, the poisoned muscle resynthesizes phosphocreatine and performs work. Such muscles contract as long as phosphocreatine is available, even though lactic acid is not formed. It is apparent, therefore, that the hydrolysis of phosphocreatine is a prerequisite to muscular contraction, and that energy-rich phosphate bonds of phosphocreatine represent a reservoir of energy.

The distribution of phosphates in resting and in fatigued muscle is given in Table 62. In resting muscle, about 80 per cent of the creatine exists as phosphocreatine and 20 per cent is free; in fatigued muscle, the free fraction is increased markedly at the expense of phosphocreatine. In

brain and nerves, only 45 per cent of the creatine is present as phosphocreatine; but this fraction is hydrolyzed on stimulation and nerve function is depressed when the phosphocreatine is decreased. Energy from the aerobic oxidation of carbohydrate and fat, and from anaerobic glycolysis, is used for the resynthesis of phosphocreatine, adenosine triphosphate, and glycogen.

TABLE 62

PHOSPHATES OF MUSCLE

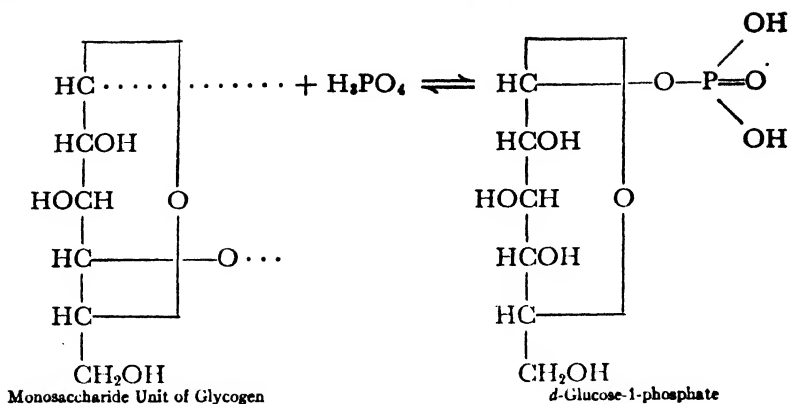
(As Mg. Per Cent P)

	At Rest	FATIGUED
Inorganic phosphate	18	60
Phosphocreatine	65	10
Pyrophosphate (chiefly adenosine triphosphate)	30	30
Adenylic acid, etc.	15	15
Hexosemonophosphate	15	15

During the carbohydrate tolerance test, the serum inorganic phosphorus of normal persons decreases from 1 to 1.5 mg. per cent within one and one-half hours and returns to the fasting level by the fifth hour. The urinary phosphate also decreases, owing to accelerated phosphorylation in the tissues. Any agent which stimulates glucose assimilation increases the movement of inorganic phosphate to the tissues. Insulin is necessary for the phosphate assimilation which accompanies glycogenesis. The sugar phosphate concentration of muscles poisoned with iodoacetate increases on stimulation. A reverse transfer of inorganic phosphate from the tissues to the blood and urine occurs as phosphocreatine is hydrolyzed, during anaerobic contraction of muscle.

FATE OF HEXOSEPHOSPHATE

The formation of *hexosemonophosphate* is a preliminary reaction which precedes oxidation, glycolysis, and alcoholic fermentation. The phosphate is usually formed biologically from glycogen or glucose; phosphorylation of glucose, fructose, or mannose requires the enzymatic factor known as *hexokinase*. This enzyme, found in brain, heart, kidney, liver, and yeast, catalyzes the formation of glucose-6-phosphate. Hexokinase is inhibited by *l*-glycerose and is reactivated by pyruvic acid; renal hexokinase is inhibited by phlorhizin. Skeletal muscle contains a *myokinase*, which accelerates the conversion of adenosine diphosphate to adenosine triphosphate and adenylic acid, thus aiding the phosphorylation of hexoses. Myokinase is activated by magnesium cations. Glycogen is phosphorylated at carbon 1 of its anhydroglucose unit by *polysaccharide phosphorylase* (page 307). This reaction may be represented as follows:



Phosphorolytic activity for glycogen is low in embryonic skeletal muscle, and in adult smooth muscle or denervated skeletal muscle. In tissues, glucose-1-phosphate changes rapidly to an equilibrated mixture of the isomeric glucose-6- and fructose-6-phosphates owing to the action of *glucophosphomutase*, a protein-magnesium complex (Fig. 7, page 316). The enzyme, *isomerase*, catalyzes the interconversion of the two 6-phosphates. Galactose-1-phosphate and fructose-1-phosphate appear in the liver and intestinal mucosa after oral administration of the corresponding sugars. In yeast, mannose and trehalose phosphates are formed. Hexosemonophosphate is aerobically oxidizable, as shown at the right of Figure 7. In the liver, the enzyme *glucophosphatase* hydrolyzes glucose-6-phosphate to glucose and inorganic phosphate. In other tissues, hexosemonophosphate can be further phosphorylated to a stabilization product, fructosediphosphate or *hexosediphosphate*, which is in enzymatic equilibrium with the monophosphate. The diphosphate does not accumulate in intact animal cells; but it appears in place of lactic acid in muscles which are poisoned with fluoride. Hexose-6-phosphate also increases in tissues poisoned with fluoride or iodoacetate.

Under anaerobic conditions the phosphorylation of glucose accelerates fragmentation into trioses. This fission is effected by the enzyme, *aldolase* or *zymohexase*, whose concentration in muscle cells is approximately 135 mg. per cent. One molecule each of triose and triosephosphate is thus formed from one molecule of hexosemonophosphate. By further phosphorylation of the triose, or alternately through hexosediphosphate, two molecules of triosephosphate are produced anaerobically from a unit of glycogen or a molecule of hexose. This second phosphorylation requires 14 Cal. per mol of hexose. Triosephosphate is an equilibrium mixture of *d*-glycerose-phosphate and dihydroxyacetone-phosphate. Anaerobically, two molecules of triosephosphate undergo dismutation (simultaneous oxidation and reduction) to form phosphoglyceric and *l*-glycerophosphoric

acids, the latter originating from dihydroxyacetone-phosphate. Since 14 Cal. are generated in this reaction, the conversion of hexosemonophosphate to the phosphate acids is thermoneutral. Glycerophosphoric acid and its hydrolytic product, glycerol, are important in lipide and phosphorus metabolism. Phosphoglyceric acid is a precursor of pyruvic acid; the latter is formed by a two-stage reaction, which involves dehydration and rearrangement by *enolase* (a protein-magnesium complex), and hydrolysis by a fluoride-sensitive phosphatase, with the liberation of 8.3 Cal. per mol. The liberated inorganic phosphate then combines with adenylic acid to resynthesize adenosine triphosphate. Pyruvic acid can react with glycerophosphoric acid to produce one molecule each of triose and lactic acid. The triose re-enters the cycle, so that finally two molecules of lactic acid are produced anaerobically from the glycogen unit or molecule of hexose. Lactic acid formation from pyruvic acid liberates another 8 Cal. per mol; hence, the entire anaerobic glycolytic cycle generates $2 \times (8.3 + 8)$ or 32.6 Cal.

The hexosemonophosphates are important in the intestinal absorption of sugars, the reabsorption of sugars by the renal tubules and calcification of bone (page 589).

FERMENTATION

Alcoholic fermentation, another anaerobic process which utilizes cellular glycogen, is subject to the Pasteur effect, and is inhibited by iodoacetate. Yeasts, fungi, and certain bacteria and plants can oxidize lactic acid to pyruvic acid, and can convert these acids to acetaldehyde and carbon dioxide. The acetaldehyde undergoes dismutation with triosephosphate to form ethyl alcohol and phosphoglyceric acid; the last-named substance then re-enters the glycolytic cycle (Fig. 7, page 316). When sulfite is present, it combines with the acetaldehyde; the hydrogen, which would otherwise unite with the acetaldehyde, is transferred to the triosephosphate to form glycerol. Glycerol also accumulates in the presence of alkali because, under these conditions, the acetaldehyde is dismuted to one molecule each of ethyl alcohol and acetic acid (the Cannizzaro reaction).

Animals and plants (including fungi) form citric acid from carbohydrates; the succinic acid produced by cells probably originates partly from carbohydrate and partly from an amino acid (aspartic acid). Muscle contains 17 mg. per cent succinic acid. Other acids and alcohols derived from carbohydrates by bacterial fermentation have been mentioned on page 299. Individual peculiarities of carbohydrate catabolism in various micro-organisms serve as useful criteria for their classification.

DEHYDROGENATION

The specific dehydrogenases concerned in the dismutation of triosephosphate, glycerophosphate, and pyruvic acid require cozymase I and yellow enzyme (Fig. 7, page 316). Iodoacetate inactivates these dehydro-

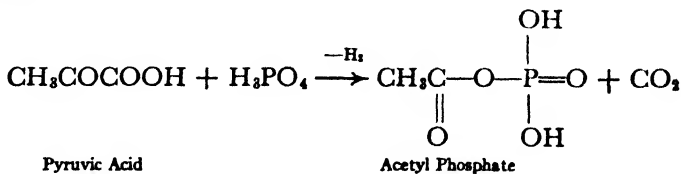
genases, and inhibits glycolysis. Iodoacetic acid reacts with the active sulfhydryl radicals of the dehydrogenases, as follows:



Glutathione prevents the inhibitory effects of iodoacetate, but it is not necessary for the normal conversion of glycogen to lactic acid. It does accelerate the production of lactic acid from methylglyoxal ($\text{CH}_3 \cdot \text{CO} \cdot \text{CHO}$), but this reaction is not regarded as important in glycolysis.

Cozymase and *reduced cozymase* form an oxidation-reduction system which is coenzymic to the specific dehydrogenases and allows transfer of hydrogen from carbohydrate intermediates to the yellow enzymes. (See pages 101 and 102 for a discussion of these carriers and their relations to nicotinic acid and riboflavin.)

Thiamin is necessary for the normal oxidation of glucose, lactic acid, and pyruvic acid in tissues. Deficiency of this vitamin causes accumulation of both acids in the blood and in the tissues. (Human blood normally contains 15 ± 5 mg. per cent lactic acid, and 0.85 ± 0.35 mg. per cent pyruvic acid.) Thiamin unites with pyrophosphate to form cocarboxylase, which accelerates decarboxylation and dehydrogenation of the acids mentioned. Fluoride, iodoacetate, and cyanide inhibit these effects. The ordinary lactic acid dehydrogenase of tissues converts lactic acid to pyruvic acid. In Figure 7, page 316, note that pyruvic acid can be directly decarboxylated to acetaldehyde by the enzyme carboxylase; or it can be reduced to lactic acid which is then oxidatively decarboxylated to acetaldehyde. The latter process appears to be important, biologically, for the following reasons: (1) Thiamin is concerned in an oxidative decarboxylation. (2) While pyruvic acid can be decarboxylated, in acid solution, to yield acetaldehyde and carbon dioxide, only lactic acid can be converted to acetaldehyde by oxidative decarboxylation; the latter converts pyruvic acid to acetic acid or acetyl radical. Certain bacteria produce acetyl phosphate by dehydrogenation of pyruvic acid in the presence of inorganic phosphate:



The acetyl phosphate contains an energy-rich phosphate bond, and it can transphosphorylate adenylic acid to form adenosine triphosphate. (3) When lactic acid, with radioactive C^{11} in the carboxyl radical, is fed to rats, a considerable fraction of the C^{11} atoms appears in the exhaled carbon

dioxide. The non-oxidative decarboxylation of pyruvic acid is a relatively unimportant process in animals and in certain micro-organisms which lack carboxylase. Cocarboxylase accelerates the synthesis of carbohydrate, acetoacetate, acetylcholine, citrate, ketoglutarate, oxaloacetate, and succinate, since these are reactions which involve pyruvate condensation.

It has been reported that biotin and pantothenic acid accelerate oxidation of lactic and pyruvic acids in bacteria and tissues, but the mechanism of their action is uncertain.

The principal aerobic oxidation of carbohydrate follows the paths indicated at the right of Figure 7, page 316. Hexose and hexosephosphate are dehydrogenated to gluconic and phosphogluconic acids, respectively, and these undergo further dehydrogenation. Gluconic and keturonic acids are characteristic carbohydrate oxidation products of fungi and acetic bacteria (*Bact. aceti*, *Bact. gluconicum*, *Bact. xylinum*). It is evident that anaerobic formation of pyruvate is not required for all types of carbohydrate utilization. Dehydrogenases, cozymase, yellow enzymes, and cytochrome are the active agents in aerobic carbohydrate oxidation; inhibition of their activity requires large concentrations of iodoacetate. During aerobic oxidation, 680 Cal. are liberated from each mol of glycogen unit; carbon dioxide and water are the final metabolites. There is increasing evidence that carbon dioxide or bicarbonate activates aerobic metabolism of carbohydrate in animals and micro-organisms (page 281). In fact, carbon dioxide is necessary for the growth of a number of bacteria, protozoa, and fungi.

THE MUSCULAR MACHINE

When muscles contract isometrically, the energy is liberated entirely as heat; but when the muscles shorten, 25 to 30 per cent of the energy appears as mechanical work. The muscle globulin, myosin, which represents 40 to 55 per cent of the muscle protein, is important in this conversion. It is concentrated in the fibrillae as the anisotropic substance. The fibrillar myosin micelles form a very elastic, thixotropic gel. At rest, the micelles show parallel orientation; but during muscular contraction, this arrangement is distorted by a helical contraction of the myosin molecules. Myosin is also an adenosine triphosphatase; it is activated by calcium ions, and inhibited by magnesium cations or by oxidation of its —SH radicals. Another fibrous protein, termed actin, is associated with myosin and adenosine triphosphate to form the muscular element which contracts in the presence of potassium salt. Adenosine triphosphate is present largely in the isotropic segments of resting muscle. In working muscle it enters the contractile anisotropic segments, where the calcium and magnesium are located. During normal muscular contraction the myosin micelles do not swell, and acidity is of little significance since the pH remains rather constant. When the oxygen supply fails, acids accumulate and accelerate the denaturation of the myosin near its isoelectric point (pH 5.1). In its

early stages this condition, known as *rigor*, is reversible by oxygen. The duration of rigor is short, and its tension is minimal when the dying cell contains little muscle glycogen (for the production of lactic acid). Heat rigor is due to heat coagulation of the myosin.

INTERCONVERSION OF SUGARS

Living cells can manufacture the mannose, galactose, glucosamine, galactosamine, glycuronic acid, and galacturonic acid units of prosthetic polysaccharides, also the ribose and deoxyribose of the nucleic acids and nucleotides. These substances are synthesized from glucose, trioses, and lactic acid. The active mammary gland secretes lactose and it synthesizes the galactose unit readily from lactic acid or from glucose, by converting one half of the latter into triose metabolites. The galactose is united with glucose to form lactose, within the gland; intravenously injected lactose cannot be used. During very rapid secretion, the mammary gland can assimilate glucose rapidly enough to produce hypoglycemia. Removal of the glands from a lactating female is followed by hyperglycemia. The mammary gland is the only tissue known to synthesize galactose rapidly, and it is, therefore, important that infants ingest some lactose or galactose to allow optimal synthesis of cerebrosides. The galactose unit of cerebrosides is derived, at least partly, from dietary lactose; the administration of this sugar to young rats has been shown to accelerate myelination. Carbohydrates are readily converted to lipides and to certain amino acids (pages 228 and 417).

EXCRETION OF SUGARS

The daily urine of normal human adults contains approximately 140 mg. of glucose, and 700 ± 500 mg. (in terms of glucose) of non-fermentable reducing substances. These non-fermentable substances have been designated, collectively, as the sugar of normal urine; they include phenols, ascorbic acid, glycuronides and unknown carbohydrates which resemble ketoses in their resistance to bromine oxidation. A fairly constant fraction of the sugar of normal urine is endogenous in origin; the remainder consists of foreign sugars ingested in toasted cereal foods, fruits, commercial syrups, and so forth. Since the reducing substances of normal urine are equivalent to less than 0.1 per cent glucose, Benedict's test is negative unless the urine is abnormally concentrated (as in oliguria).

Carbohydrates are excreted indiscriminately by the kidney glomeruli, but the utilizable sugars and ascorbic acid are actively reabsorbed through the epithelium of the proximal convoluted tubules. Glucose, galactose, mannose, fructose, and xylose are reabsorbed at decreasing rates in the order named. Although this order differs from that cited for intestinal absorption, both processes are known to depend on the activity of phosphory-

lases and phosphatases in the living epithelia. The renal tubules reabsorb more than 99.8 per cent of the glucose contained in the normal glomerular filtrate. In normal men, the maximal rate of tubular reabsorption (T_m) for glucose is approximately 345 mg. per minute. This rate is attained at a blood sugar level between 350 and 700 mg. per cent. Reabsorption of glucose from the glomerular filtrate is incomplete when the blood sugar rises above a characteristic level known as the *renal threshold*. In normal humans, this level averages about 150 mg. per cent; but it can be anywhere between 115 and 215 mg. per cent. The threshold is quite constant and characteristic for the individual. In narcosis, arteriosclerosis, nephritis, or diabetes in elderly patients, the threshold may be as high as 350 to 400 mg. per cent.

When the blood sugar rises above the threshold value, the tubules cannot reabsorb sufficient glucose to prevent the excretion of abnormally large quantities in the urine. Such *glycosuria* is readily detected by the ordinary qualitative sugar reagents. The glucose threshold is affected by metabolic conditions in the kidney and by the duration of the hyperglycemia. Cyanide can cause glycosuria by interfering with respiration (and reabsorption) in the renal tubular tissue. Glycosuria is intensified as the blood sugar rises above the threshold; but only a fraction of the excess is excreted, inasmuch as the liver and general tissues are simultaneously withdrawing glucose from the blood. Glucose utilization by normal tissues increases with the blood glucose concentration until a level of approximately 1,000 mg. per cent is reached. Since insulin stimulates glycogenesis, it increases the fraction of sugar which is utilized, and decreases glycosuria correspondingly. Glycosuria frequently lags behind hyperglycemia, just as glycogenesis is delayed during the carbohydrate tolerance test. Glycosuria is often associated with glycogenolysis, gluconeogenesis, and ketogenesis; when the concentration of glucose in the urine rises to 4 per cent, or more, marked polyuria and dehydration occur.

Glycosuria is caused by excess adrenaline, thyroxine, diabetogenic factor, or by any other factor which leads to hyperglycemia above the threshold value. *Alimentary glycosuria* results from the ingestion of large quantities of utilizable carbohydrate. It is not often encountered after average meals, unless these are administered during the late afternoon. Less than 10 per cent of normal persons exhibit glycosuria in the carbohydrate tolerance test. Glucose can be injected intravenously into humans and dogs at a rate of 0.85 gm. per kg. of body weight, per hour, without glycosuria; a more rapid influx leads to glucose excretion. The maximum injection tolerated by a 65 kg. man is, therefore, 55 gm. of glucose per hour. Only one fourth of this sugar is oxidized during the injection; the remainder is stored as glycogen and fat.

Threshold values for glucose are not applicable to other sugars. The threshold for fructose is believed to be in the vicinity of 120 mg. per cent, but 10 per cent of normal individuals exhibit fructosuria when 100 gm. of

fructose are given orally. There are no significant renal thresholds for galactose, mannose, pentoses, disaccharides, or inulin. These sugars are excreted when only small quantities are present in the blood. After oral administration, they are partially converted to glucose in the liver. The assimilation limits of sugars have been considered on page 302.

The urinary sugar excretions, or *melliturias*, of greatest clinical interest include glucosuria, lactosuria, pentosuria, and fructosuria; glucosuria is by far the most frequent. Abnormal concentrations of urine sugar are qualitatively detected by Benedict's reagent, which gives a greenish precipitate (++) in one minute with 0.1 to 0.2 per cent glucose, a yellow precipitate (+++) in fifteen seconds with 0.2 to 1 per cent glucose, and an immediate red precipitate (++++) with more than 1 per cent of glucose. The most convenient and reliable method for the quantitative determination of urine sugar is Sumner's method; it is much less sensitive than copper methods to such urinary nitrogenous reducing substances as creatinine, uric acid, and allantoin. Lactosuria and pentosuria are characterized by non-fermentability of the urine sugar. To determine the true glucose content of urine requires incubation of the sample with washed yeast for forty-five minutes, and determinations of reducing sugar before and after the fermentation. Measurement of the carbon dioxide evolution is unreliable, owing to the frequent contamination of baker's yeast with bacteria which are capable of fermenting a variety of substances. Glucose may also be distinguished from other sugars by the osazone test. Here, technique is important. Concentrated urines, which have high concentrations of urea, give crystals of phenylsemicarbazide that are easily confused with phenylglucosazone. Pentosuria is detected by Bial's test and by the rapid destruction of the reducing activity of the urine by hydrogen peroxide. Fructosuria is detected by Seliwanoff's test and by marked levorotation of the urine.

Phlorhizin Glycosuria

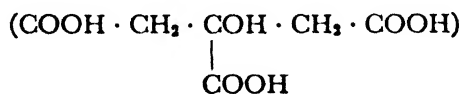
Subcutaneous injection of the glucoside, phlorhizin, inhibits tubular phosphorylation and reabsorption of glucose and causes marked glycosuria. Phlorhizin accumulates in the epithelium of the proximal tubule; it is excreted as a phenolic glucuronide which reacts like acetoacetic acid in Gerhardt's test. The kidneys of phlorhizinized animals excrete various soluble sugars at almost identical rates. Since the blood sugar remains normal, or slightly subnormal, the glycosuria represents a lowered threshold. The drug produces little disturbance of carbohydrate metabolism in nephrectomized animals. The fundamental process in phlorhizin diabetes is a carbohydrate starvation, resulting from excessive loss of glucose in the urine. The respiratory quotient is low (0.7), protein is rapidly transformed to glucose and urea, and large amounts of lipides are oxidized with concomitant ketosis and ketonuria. When sufficient glucose or insulin is

injected into these animals, carbohydrate is diverted to the tissues and oxidized in normal quantities; as a result, ketosis disappears, gluconeogenesis diminishes, and the urinary nitrogen excretion decreases.

Phlorhizin glycosuria has been a very useful experimental procedure for investigating gluconeogenesis; all substances which can form glucose do so at maximal rates in starving phlorhizinized animals, and the glucose formed appears almost quantitatively in the urine. Phlorhizinized animals have been used to demonstrate that protein is an available gluconeogenic substance, and that the diabetogenic factor, thyroglobulin, and corticosterone stimulate gluconeogenesis. During starvation, both phlorhizinized and depancreatized animals at times exhibit a urinary G/N ratio (gm. glucose/gm. nitrogen) of approximately 3.65. Since protein contains about 16 per cent nitrogen, a G/N ratio of 3.65 represents the conversion of 3.65×16 , or 58 per cent, of muscle protein to glucose. Conversions of other proteins to glucose, in phlorhizinized animals, are as follows: wheat gliadin, 80 per cent; edestin and gelatin, 65 per cent; soy bean glycinin, 61 per cent; egg and serum albumins, 55 per cent; zein, 53 per cent; and casein, 48 per cent. These yields correspond to the distribution of gluconeogenic amino acids (Table 66, page 355). The 3-carbon keto acid fragments of amino acids are important in gluconeogenesis. Thus, glycine and the 3-carbon acids are quantitatively converted to glucose, while only three carbons of arginine, ornithine, proline, hydroxyproline, valine, aspartic acid, and glutamic acid are convertible. In starving phlorhizinized animals, adrenaline injection increases the sugar excretion by stimulating tissue glycolysis and hepatic synthesis of glucose from lactic acid. The G/N ratio has been used to estimate the severity of diabetes; the nearer the ratio approaches 3.65, the more complete is the diabetic condition.

METABOLISM OF ACIDS DERIVED FROM CARBOHYDRATES

Mammalian tissues rapidly oxidize lactic acid, glyceric acid, and citric acid



Smaller quantities of acetic, gluconic, malic ($\text{COOH} \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{COOH}$), pyruvic, saccharic, succinic ($\text{COOH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$), and tartaric ($\text{COOH} \cdot \text{CHOH} \cdot \text{CHOH} \cdot \text{COOH}$) acids are also utilized; but oxalic acid ($\text{COOH} \cdot \text{COOH}$) is very resistant to oxidation. With the exception of saccharic and tartaric acids, all of the acids mentioned are found in small quantities in tissues. They are the products of side reactions of carbohydrate and protein metabolism. Lactic, glyceric, citric, malic, pyruvic, propionic, butyric, gluconic, succinic, and tartaric acids form

liver glycogen in decreasing amounts, approximately in the order named. The first five are also partially interconvertible in the body. Experiments with isotopic lactic acid which contains C^{11} isotope in the carboxyl radical have demonstrated that the formation of glycogen from this acid involves oxidative processes. When fed to rats, less than 2 per cent of the radioactive carbon is found in the newly formed hepatic glycogen, and 20 per cent appears in the exhaled carbon dioxide. Since one third of the lactic acid goes to liver glycogen, it must be oxidatively decarboxylated, prior to glycogenesis. C^{11} ingested as sodium bicarbonate is partially incorporated into the hepatic glycogen, probably by reversal of decarboxylation reactions. Carbon dioxide unites with pyruvic acid to form oxaloacetic acid ($COOH \cdot CH_2 \cdot CO \cdot COOH$), and the latter can be converted to succinic acid, citric acid, malic acid, α -ketoglutaric acid ($COOH \cdot CH_2 \cdot CH_2 \cdot CO \cdot COOH$), and other intermediates. Radioactive carbon dioxide can be used to demonstrate the formation of the added carboxyl radical. Oxaloacetic carboxylase is the enzyme responsible for the fixation of carbon dioxide by pyruvic acid; it is activated by manganous cations. Cocarboxylase accelerates these syntheses, which take place in fungi, bacteria, and mammalian liver, kidney, and cardiac (but not skeletal) muscle. The liver can form acetoacetic acid from pyruvic acid, and the heart and kidney can produce citric acid from acetoacetic and oxaloacetic acids.

Oxalic acid is absorbed rapidly from the intestine. Owing to its stability, its ability to combine with ionized calcium, and its inhibition of carbohydrate catabolism, oxalic acid is the most toxic of the sugar acids mentioned above. As little as 5 gm. *per os* has proved fatal to human beings. Oxalic acid is excreted in the urine, partly as oxaluric acid; the normal daily output is from 10 to 50 mg., derived largely from the endogenous metabolism of carbohydrate and protein, and a smaller amount from food oxalates (Table 15, page 75). Tartaric acid is absorbed more slowly and is less toxic than oxalic acid, but it resembles the latter in other respects. Excessive quantities of these acids tend to produce kidney damage.

Malic and citric acids are, quantitatively, the most important fruit acids of human diets. Citric acid preponderates in apricots, beets, blueberries, cantaloupes, cranberries, currants, figs, citrus fruits, loganberries, pineapples, potatoes, raspberries, strawberries, and tomatoes. Malic acid is the principal organic acid in apples, blackberries, carrots, celery, cherries, cucumbers, grapes, lettuce, mushrooms, okra, onions, parsnips, plums, prunes, quince, rhubarb, squash, and turnips. (Grapes and tamarinds contain considerable tartaric acid.) Dietary citrates and malates are absorbed rapidly in the intestine and utilized as energy sources. Approximately 2 mg. per cent citric acid are present in normal serum; the blood level is elevated by parathormone, nephrectomy, obstructive jaundice, and severe liver disease. Embryonic tissue, tumors, hair, skin, teeth, and the hard substance of bone contain noteworthy concentrations of citrate.

The bone citrate is lowered by vitamin D deficiency. A daily intake of 40 gm. of citric acid is oxidized completely, and 5 gm. of sodium citrate are well tolerated intravenously (as in blood transfusions). The acid is synthesized continuously in the body from pyruvic, acetoacetic, and oxaloacetic acids. Citric acid is removed rapidly from the blood by the liver and the kidney; the urine of adults contains from 0.2 to 1 gram of the acid daily. The excretion of citrate is enhanced by alkalosis, ingestion of sodium bicarbonate, and injection of sodium succinate, lactate, malate, or pyruvate. Since these procedures do not raise the blood citric acid level, it is believed that the kidney plays an active role both in the catabolism and in the excretion of the acid. Citric acid excretion is decreased in thiamin deficiency.

Metabolism of Glycuronic Acid

This reducing acid is formed principally in the liver, perhaps from pyruvic acid or by the oxidation of glucose-1-phosphate, since its formation (glycuronogenesis) is inhibited by fluoride, cyanide, and iodoacetate. The formation of glycuronic acid is stimulated by a variety of foreign substances, such as phenols, alcohols, ketones, aldehydes, hydrocarbons, aromatic acids, and compounds which can be either oxidized or reduced to these substances. Liver slices produce conjugated glucuronides from these drugs much more actively than do kidney slices or intestinal mucosa. Glycuronide production complements hippuric acid formation and sulfate conjugation in the detoxication of drugs and poisons. Glycuronogenesis is increased when glycine is deficient, or when sources of sulfate are depleted.

A partial list of drugs which stimulate glycuronogenesis is given in Table 63. In the liver, glycuronic acid unites with these foreign substances (potential aglycones) to produce *glycuronides* (*glycuronates*), and with aromatic acids to form glycuronic acid esters. The two types of glycuronic acid conjugates are illustrated in Table 63. The glycuronides are stronger acids than the corresponding acid aglycones.

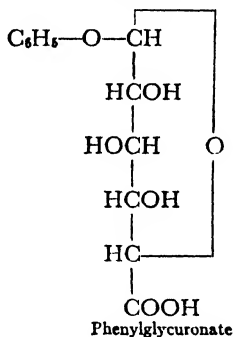
Normal animals can form glycuronides in large quantities. (However, glycuronic acid, or *d*-arabinose, is a dietary essential for normal growth of chicks.) The diabetic animal can synthesize some glycuronic acid from gluconeogenic amino acids; injection of insulin markedly increases glycuronogenesis in diabetic animals although it has less effect in normal animals. Ketosis, and the injection of adrenaline, tend to repress the formation of glycuronic acid. Glycuronic acid is not a glycogen-former; a small fraction of ingested glycuronic acid is oxidized; the remainder is excreted. Ingested glycuronic acid cannot be used for conjugation, neither do animals convert phenyl glucosides to glycuronides. Menthol glycuronate is almost completely oxidized; and in man glycuronic acid monobenzoate is converted to hippuric acid. Mammalian tissues contain a β -glycuronidase; activity of this enzyme in the uterus is increased by estrogens. Pentosuric patients excrete *l*-xyloketose after the administra-

TABLE 63

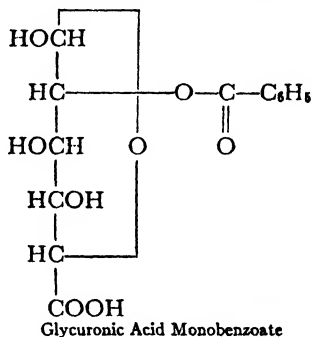
DRUGS WHICH STIMULATE GLYCURONOGENESIS

Acetanilide	Chloroform	Methyl salicylate	Santal oil
Acetphenetidin	Creosote	Morphine	Santonin
Aminopyrine	Cresol	Naphthalene	Sex hormones
Antipyrine	Eucalyptus oil	Naphthol	Skatoxyl
Acetylsalicylic acid	Fennel oil	Phenol	Sulfapyridine
Balsams	Guaiacol	Phenylacetic acid	Terpene hydrate
Benzene	Hexylresorcinol	Phlorhizin	Thuja oil
Benzoic acid	Indoxyl	Pyramidon	Thymol
Borneol	Isobutyl alcohol	Resorcinol	Tribromethanol
Camphor	Isoamyl alcohol	Saccharin	Turpentine
Chloral hydrate	Juniper oil	Salicylic acid	Vanillin
Chloralose	Menthol		

GLYCURONIDE



GLYCURONIC ACID ESTER



tion of glycuronic acid or some of the drugs listed in Table 63. These individuals have an abnormal enzyme system which decarboxylates glycuronic acid and transforms it to the xyloketose.

Human blood normally contains about 7.5 mg. per cent of glycuronic acid in the form of non-protein glycuronides. The 150 ± 80 mg. of glycuronic acid in daily normal urine represent the detoxication of phenols and sex hormone metabolites (estriol, pregnandiol, etc.). Glycuronides are usually levorotatory and non-reducing, but in urine they are at times sufficiently hydrolyzed to reduce copper reagents. Unhydrolyzed glycuronic acid esters can reduce these reagents. (See formula, Table 63.) Glycuronide excretion can be distinguished from glycosuria by fermentation and by the naphthoresorcinol test.

METABOLISM OF ASCORBIC ACID

This vitamin is a typical plant metabolite; with the exception of the postnatal monkey, guinea pig, and human being, it is manufactured by

animals in sufficient quantity to supply their biological needs. Ascorbic acid is stereochemically related to *l*-gulose and *l*-sorbose, but these sugars are not found in mammalian tissues. Although ascorbic and dehydroascorbic acid form an oxidation-reduction system, animal tissues contain mostly ascorbic acid (page 105). Injected dehydroascorbic acid is partly converted to ascorbic and diketogulonic acids in animals. Ascorbic acid is stored in very limited amounts by man, chiefly in glands such as the adrenals, corpus luteum, thymus, pituitary, pancreas, ovary, testis, spleen, and liver. Any excess is excreted in the urine at a rate which depends on the blood level and the saturation of the tissues. The threshold for ascorbic acid is about 1.5 mg. per cent, and the maximal rate of tubular reabsorption (T_m) is approximately 2.2 mg. per minute. Normally, 1.2 ± 0.6 mg. per cent is found in the plasma and the cerebrospinal fluid; the leukocytes contain more than 25 mg. per cent. In late pregnancy, the blood ascorbic acid content is frequently low; fetal blood contains from two to three times as much ascorbic acid as does maternal blood. Injection of corticotropic hormone decreases the ascorbic acid concentration in the adrenal glands. The vitamin C content of human tissues decreases with age. The daily excretion by the normal adult is from 10 to 40 mg.; in scorbutic conditions, the blood level and the urinary excretion are subnormal. (See page 665 for further discussion of ascorbic acid metabolism.)

INOSITOL

This cyclitol was formerly known as muscle sugar. It is found in free form in all animal tissues, the largest concentrations being in muscle, as phytic acid in chicken and turtle blood, and as the phospholipide, lipositol, in mammalian tissues. Inositol is widely distributed in plants as the hexaphosphate ester, phytin, which is digested with difficulty by man (page 594). Both phytin and inositol have laxative effects. Yeasts and bacteria can convert inositol to carbohydrate metabolites. Free inositol increases in tissues during embryonic development; it decreases markedly in brain and muscle during later growth of the animal. Total inositol is constant in the developing chick embryo. Normal urine does not contain inositol; but excretion of this cyclitol occurs during marked polyuria of any type. *i*-Inositol is the vitamin factor known as Bios I.

PATHOLOGY

"When we say that worry will bring about certain bodily phenomena, we use the word worry to denote a state of the entire person (both body and mind) and do not exclude the bodily processes which are necessarily involved." — MORRIS R. COHEN

HYPERGLYCEMIA

Fasting hyperglycemia is the result of delayed assimilation of glucose by the liver and the general tissues. It is an important symptom of dia-

betes mellitus; but it is also encountered in acidosis, acute infections, chronic arterial hypertension, and hyperpituitarism, and, occasionally, in hyperthyroidism, renal failure, and Hand-Schüller-Christian disease. The relative infrequency of hyperglycemia in well developed hyperthyroidism is due to increased muscular activity, increased carbohydrate oxidation, and depletion of hepatic glycogen. Hyperglycemia can be produced by the excessive secretion of adrenaline which results from adrenal tumors, apoplexy, brain tumors, concussions, encephalitis, or other intracranial pathology; or by asphyxial conditions caused by anesthetics, carbon monoxide poisoning, severe hemorrhage, traumatic shock, or convulsive states (eclampsia, epilepsy, tetany, tetanus, uremia, etc.). The blood sugar rises only 8 mg. per cent for each ounce of ether, skillfully administered to non-diabetic patients; the increase is much greater in diabetics. Morphine raises blood sugar from 20 to 40 mg. per cent. Anesthetics (especially chloroform), barbiturates, and morphine very rapidly deplete the hepatic glycogen in patients who have liver disease. Anesthetics and hypnotics stimulate adrenaline secretion, and depress carbohydrate oxidation in the tissues. Chloralose and amytal cause the least disturbance of carbohydrate metabolism. Purine diuretics and quinine can produce hyperglycemia.

Diabetes mellitus differs from the other conditions mentioned, in that it does not require a store of hepatic glycogen to produce fasting hyperglycemia. The height of the fasting blood sugar is only partially related to the severity of the diabetes; mild cases, and those complicated by acute liver disease or by pregnancy toxemia, frequently show only slight hyperglycemia. In such cases, the carbohydrate tolerance is determined in order to establish a diagnosis. In advanced diabetes, the fasting blood sugar is usually from 180 to 400 mg. per cent, while in coma the level may exceed 700 mg. per cent. Repeated fasting blood sugar values in excess of 135 mg. per cent are highly suggestive of diabetes. If acidosis, acute infection, hyperpituitarism, hypertension, hyperthyroidism, nephritis, or other hyperglycemic complications are present, 150 mg. per cent is considered to be a better differential value.

HYPOGLYCEMIA

The fasting blood sugar may be as low as 40 mg. per cent in spontaneous hyperinsulinism. Hyperinsulinism is usually the result of hyperplasia or adenoma of pancreatic islet tissue, or of insulin overdosage. There is a physiological hypoglycemia in starving persons, and in certain normal persons three to four hours after meals; this tendency is more pronounced in children. The infants of inadequately treated diabetic mothers weigh more than normal infants, and they exhibit an excessive secretion of insulin, stimulated by prolonged hyperglycemia *in utero*. The hypersecretion continues for some time after birth, and severe hypoglycemia may

develop unless glucose is administered. Slight hypoglycemia occurs in progressive muscular atrophy, status lymphaticus, glycogen disease, hypopituitarism (advanced acromegaly and Fröhlich's syndrome), hypoadrenalism (Addison's disease), and severe burns, and occasionally during lactation and hypothyroid conditions (myxedema, cretinism, and following thyroidectomy of patients who have received insufficient carbohydrate prior to operation). In terminal adrenal failure, the blood sugar may fall below 30 mg. per cent; and in pituitary cachexia (Simmonds' disease), marked hypoglycemia occurs. Eighty per cent or more of the liver of a normal animal must be removed to produce pronounced hypoglycemia. Hence, this condition may be anticipated in hepatic disease only when the liver has undergone diffuse damage, as in the terminal stages of cirrhosis, fatty infiltration, hepatic carcinoma, syphilis, or yellow fever, or in acute atrophy and necrosis caused by arsphenamine, carbon tetrachloride, chloroform, or cinchophen. Hypoglycemia can also be produced by rapid exhaustion of liver glycogen during anesthesia, convulsions, or biliary surgery. A high carbohydrate diet or intravenous glucose therapy is, therefore, important before operation.

Hypoglycemic Shock

Hypoglycemia below 45 mg. per cent often causes marked symptoms, especially when the blood sugar falls rapidly, although some patients are remarkably insensitive to low blood sugar levels. Symptoms of acute hypoglycemia in human beings include fatigue, weakness, drowsiness, anxiety, hunger, tremor, excessive perspiration, vasomotor disturbances, restlessness, loss of inhibitions, diplopia, headache, confusion, hallucinations, amnesia, aphasia, catatonia, vertigo, and coma. The pulse rate is increased, the plantar response (Babinski sign) is positive, the pupils are dilated, and the eyeballs are firm. Some of the symptoms are ascribed to adrenaline liberation. Convulsions of central nervous system origin may appear and last for hours; they disappear under anesthesia. Coma may develop with or without previous convulsions, and this condition may be mistaken for ketosis in treated diabetic patients. Differentiation of the two conditions is very important because of the danger of insulin injections during hypoglycemia. Delay in raising the blood sugar level may prove fatal, and protracted hypoglycemia in diabetic patients can cause subsequent exacerbation of the disease owing to increased hepatic glycogenolysis and ketosis. Hypoglycemia differs from the coma of ketosis by the characteristic symptoms mentioned above, by its rapid appearance, and by the absence of fever, pain, thirst, vomiting, irregular breathing, and lowered alkali reserve. There is, usually, some history of insulin administration, or of low food intake; hypoglycemic shock tends to occur about three to four hours after the administration of excess ordinary insulin, or twelve to eighteen hours following protamine-insulin. The first specimen of urine

may reduce Benedict's reagent, because of urine retention; but sugar disappears from subsequent specimens. The administration of from 5 to 10 gm. of glucose by mouth, or of a 50 per cent glucose solution intravenously, gives prompt relief in the early stages of hypoglycemic shock. Intravenous injections of citrate, dihydroxyacetone, fructose, galactose, glycerol, glycogen, maltose, or mannose are less effective; other sugars, and lactic, pyruvic, or glycerophosphoric acid are ineffective. If liver glycogen is available, the injection of adrenaline or of pituitrin aids in restoring the blood sugar. The use of repeated insulin shocks in early schizophrenia is an empirical treatment, and was originally employed to relieve the abstinence symptoms of morphine addicts. Insulin shock temporarily lowers the oxygen uptake of the brain, and increases the lactic acid and the choline esterase concentrations of the blood. In metrazol shock, the supply of oxygen to the brain is decreased by unsaturation of the arterial blood. In some patients, these asphyxial measures cause encephalopathic changes.

ABNORMAL CARBOHYDRATE TOLERANCE

The principle of the carbohydrate tolerance test has been discussed on page 303. For clinical purposes, blood samples are taken at hourly or half hourly intervals; and urine specimens representing the first two hour and the subsequent twenty-two hour periods are tested for sugar. Decreased glucose tolerance is indicated by a sustained hyperglycemia with a maximal blood sugar value above the normal peak (150 mg. per cent). The hyperglycemic index (page 304), which normally is 0, becomes 10 to 50 in moderately reduced tolerance, and exceeds 50 in severe cases. In diabetics, this index is usually inversely proportional to the blood cholesterol. The most significant and characteristic decrease in carbohydrate tolerance occurs in diabetes mellitus, owing to inhibition of glycogenesis. Both fasting and maximal blood sugar levels tend to increase in proportion to the severity of the diabetic condition. The typical diabetic tolerance curve shows a "plateau" with the hyperglycemic peak later than one hour, and a delayed decline to the fasting level after the third hour. The arterio-venous difference at the peak of the tolerance curve is markedly diminished in diabetes; the respiratory quotient also remains low, while serum phosphate shows little depression.

Tolerance curves of hyperpituitary patients resemble those of diabetics, with the exception of a more rapid return to the normal blood sugar level. The diabetic curve in the two dose tolerance test shows a characteristic sharp rise in the blood sugar level after the first hour. Diabetics do not exhibit the Staub-Traugott effect; they respond to increasing doses of glucose with a greater and more prolonged hyperglycemia, because glycogenesis in diabetic tissues cannot keep pace with the intestinal absorption of sugar.

Since abnormal hepatic function is the chief direct cause of decreased

carbohydrate tolerance, this condition also results from prolonged fasting, high fat diets, and at times from acidosis, Hand-Schüller-Christian disease, hyperadrenalism, hyperpituitarism, hyperthyroidism, infections, jaundice, yellow atrophy, or poisoning of the liver by arsphenamine, carbon tetrachloride, chloroform, or cinchophen. In chronic cirrhosis, cancer, syphilis, or passive congestion of the liver, there is usually sufficient hepatic functional reserve to produce a normal tolerance curve. The typical result in liver disease is a normal to slightly subnormal fasting blood sugar, increased maximal hyperglycemia at one hour, and a normally descending curve; only in very severe liver disease is the hyperglycemia prolonged. Moderately decreased tolerance occurs in late pregnancy (when the pituitary and thyroid glands are hyperactive), in certain cases of acute arthritis accompanied by focal infection, also in severe anemias, essential hypertension, and occasionally in nephritis, or during the menstrual period.

Increased carbohydrate tolerance is indicated by a flattened curve and a maximal hyperglycemia of less than 150 mg. per cent; this is the case in hypothyroidism (cretinism, myxedema), hypopituitarism, Addison's disease, sprue, and celiac disease. In these conditions delayed glucose absorption slows hepatic glycogenesis, and decreases the arteriovenous difference. Thyroidectomy produces no marked alteration in the tolerance curves of normal animals, although it increases the tolerance of hyperthyroid patients.

Hyperinsulinism might be expected to produce a flattened curve; but in this condition the liver is usually saturated with glycogen, and excessive temporary hyperglycemia therefore results during the tolerance test. The condition is characterized by fasting hypoglycemia, and by alleviation of acute hypoglycemic symptoms by the administration of carbohydrate. A high protein, low carbohydrate diet, similar to that used in diabetes mellitus, is best suited to the prevention of postprandial hypoglycemia in hyperinsulinism, because glucose is liberated gradually in the body through gluconeogenesis.

In infants and children with von Gierke's *glycogen disease* the fasting blood sugar level is low and does not respond normally to the injection of adrenaline. The liver, heart, and kidneys increase in size, and contain massive immobile glycogen deposits; the skeletal muscles show degenerative changes and ketonuria is present. Glycogenolysis is inhibited and the tolerance curve is flattened at a low blood sugar level.

Tolerance to Fructose and Galactose

In the clinical fructose tolerance test, 50 gm. of the sugar are administered in approximately 200 ml. of water and lemon juice. Normal persons respond with a maximal blood sugar of 130 mg. per cent (or 20 mg. per cent fructose), and the normal level is restored in from one and one-half to two hours. Decreased fructose tolerance, that is, a higher peak

and a delayed return to the fasting level, suggests the presence of hepatic insufficiency; but the response is frequently normal even in advanced chronic liver disease. Like the ordinary glucose tolerance test, and the response of the blood sugar to adrenaline, the fructose tolerance is of small clinical diagnostic value in hepatic disease.

The galactose tolerance test is believed to have somewhat greater significance. After the administration of 30 gm. galactose to males, or 40 gm. to females, the blood normally contains about 30 mg. per cent galactose at one hour, and none in two hours. However, the usual index is the urinary excretion of galactose. Hepatic insufficiency is indicated if the five hour urine specimen contains more than 3 gm. galactose. Rather extensive liver damage is necessary to cause decreased galactose tolerance. Decreased tolerance to galactose has been reported in acute parenchymatous hepatic damage, and at times in gonad dysfunction, hyperpituitarism and pregnancy. Hepatctomized animals excrete from two to three times as much galactose as do normal animals.

GLYCOSURIAS

Clinical glycosuria is usually caused by hyperglycemia, although occasionally *renal glycosuria* results from a lowered threshold, with an essentially normal blood sugar level. In renal glycosuria, the carbohydrate tolerance is normal, the urine reduces Benedict's reagent more or less constantly, and insulin has little effect upon the glycosuria. This condition accounts for approximately 0.2 per cent of clinical glycosurias; it can occur in otherwise normal persons as a benign congenital abnormality of kidney metabolism, but it is more frequent in late pregnancy, severe nephritis, and nephrosis. In kidney disease, glycosuria is sometimes associated with hyperglycemia, but when the degeneration interferes with tubular reabsorption, the kidney threshold is lowered while the blood sugar level remains normal.

Glycosurias associated with hyperglycemia occur in diabetes mellitus, and, at times, in acidosis, acromegaly, asphyxial conditions, chronic glomerulonephritis, chronic liver disease, hyperadrenalism, hypertension, hyperthyroidism, increased intracranial pressure, meningitis, pregnancy, and following anesthesia. Diabetes is the cause of only 10 per cent of clinical glycosurias in adults, whereas in children it is responsible for 30 per cent. In about one third of hyperthyroid patients, and especially those with exophthalmic goiter, glycosuria is partially related to the increased basal metabolism. In a few cases, hyperthyroidism predisposes to diabetes mellitus. Glycosuria during early pregnancy is likely to be of diabetic origin.

Considerable emphasis was formerly accorded to *emotional glycosuria*, which results from anger, anxiety, excitement, fear, pain, and the like. Asphyxia, extensive hemorrhage, emotional states, stimulation of sensory nerves, piquê (puncture of the floor of the fourth ventricle of the brain),

lesions in the hypothalamic tract, and intracranial damage produced by apoplexy, concussion, and tumors, stimulate medullary impulses which are transmitted via the splanchnic nerves to the liver and the adrenal glands. Adrenaline secretion is also stimulated by acetylcholine, morphine, nicotine, and strychnine. The resultant hyperglycemia and glycosuria are due to hepatic glycogenolysis. Emotional glycosuria is occasionally encountered in normal human beings, but is relatively infrequent in excited psychotic patients; it should not be regarded as a regular result of human emotion or excitement unless this is catastrophic, as for example, the fear of death. Emotional glycosuria is most common in elderly patients with organic disease, and in sad, dispirited, psychotic patients. Some "emotional glycosurias" are actually due to oliguria, by which the urinary concentrations of normal reducing constituents are increased; others are merely renal glycosurias.

OTHER MELLITURIAS

Fructosuria accompanies glycosuria in rare cases of severe diabetes. Even more rare is the benign congenital error of fructose metabolism termed essential fructosuria, in which there is a renal abnormality of fructose reabsorption, and diminution in fructose oxidation. Insulin has no effect on essential fructosuria.

Temporary *pentosuria* can result from the ingestion of excessive quantities of cherries, grapes, plums, prunes, or other fruits. A benign essential pentosuria, of congenital origin, constitutes about 0.1 per cent of clinical melliturias. Like diabetes, essential pentosuria has definite hereditary relations. In this condition, *l*-xyloketose is formed from glycuronic acid (page 331).

Lactosuria frequently occurs in lactating women; it is due to the diffusion of lactose from the mammary glands into the blood, and its prompt excretion in the urine. Lactose and galactose are also occasionally found in the urine of infants after excessive ingestion of lactose, or during gastrointestinal disease. Lactosuria does not have pathological significance. Rare cases of persistent *galactosuria* are found in infants in association with enlargement of the liver and spleen, anemia, osteoporosis, and arrest of growth. A lactose free diet results in some clinical improvement.

DIABETES MELLITUS

This is a fairly common disease, which is caused by deficient secretion of insulin by the β -cells of the pancreatic islands of Langerhans. Diabetes mellitus shows hereditary tendencies; it is especially prevalent among Hebrews. The condition fluctuates in severity, but clinical instances of total diabetes are rare. The diagnosis of diabetes mellitus is based on symptomatic evidence, and repeated observation of glycosuria, hyper-

glycemia, and decreased glucose tolerance. Fasting hyperglycemia, due to decreased assimilation of glucose and decreased glycogenesis by the tissues, is a constant feature of the disease. The height of the blood sugar is only partially related to the severity of the condition, which is also affected by abnormalities of the liver, kidney, and endocrine glands, and by acidosis and infections. In untreated cases, the blood sugar may rise gradually to more than 2,000 mg. per cent. If pathological changes develop in the liver, the fasting blood sugar level tends to be lowered. Paroxysmal hypoglycemia can result from severe hepatic damage, as in hemochromatosis. After hepatectomy, the blood sugar of a diabetic animal falls rapidly.

In diabetic patients the blood sugar tends to rise during the night. Alimentary hyperglycemia is, therefore, most marked following breakfast. The syndrome of diabetes develops rapidly in depancreatized cats or dogs; if untreated, these completely diabetic animals usually die in from two to three weeks. Injection of 200 mg. per kg. of alloxan is another method for producing experimental diabetes mellitus in animals. This pyrimidine, as well as alloxantin, causes selective destruction of the islands of Langerhans. The pancreas is the only organ which secretes or stores insulin in appreciable quantity, although small amounts are found in many tissues. Functional deficiency, or pathological degeneration of the islet tissue is, therefore, the fundamental cause of diabetes; acinar degeneration of the pancreas, resulting from ligation of the pancreatic duct, has no effect on insulin secretion until the islets become involved.

The totally diabetic animal loses all administered carbohydrate, together with the gluconeogenic portion of the protein. In severe cases of human diabetes, as much as from 200 to 300 gm. glucose may appear in the urine daily. In mild cases, the glycosuria is intermittent, provided the blood sugar is not maintained above the threshold. For this reason, twenty-four hour specimens of urine should always be examined. In mild or moderate cases the urine contains less than 3 per cent of glucose, while in severe cases it may rise to 8 per cent. Marked glycosuria causes an increase in urine volume to as much as several gallons daily; the polyuria causes pronounced dehydration and loss of base (particularly as sodium chloride), and these complications are enhanced by ketosis. Diabetic polyuria is unique as regards the high specific gravity of the urine, due to its sugar content. The threshold may vary in diabetes; glycosuria may disappear at times, in older patients, as the result of nephritic or arteriosclerotic complications, which can increase the threshold to levels as high as 425 mg. per cent. Polyuria and pruritis are symptoms which commonly bring the disease to the attention of the patient and the physician.

The tolerance test is of particular value in diagnosing cases of diabetes with moderate or slight hyperglycemia, or when complications obscure

the diagnosis. If decreased tolerance is not demonstrable, diabetes is not present. Tolerance is decreased by hepatic complications, and especially by ketosis, hyperpituitarism, and hyperthyroidism, which also affect the liver. The tolerance is often temporarily improved after a period of ketosis or of starvation, and after the administration of estrogen. Removal of the liver from a diabetic dog, which is treated with insulin, re-establishes the diabetic tolerance curve. Carbohydrates are better tolerated when the blood sugar is falling and hepatic glycogenesis is in progress. The diabetic R.Q. is below normal, owing to hepatic gluconeogenesis and subnormal supply of glucose to the tissues. It is not appreciably raised by adrenaline, exercise, or glucose, in contrast to the effect of insulin administration. The basal metabolic rate is occasionally low; it is restored to normal by insulin.

Ketosis, lipemia, hepatic fatty degeneration and infiltration, and other disturbances of lipid metabolism encountered in diabetic patients have been discussed on pages 37, 248 and 254. The occurrence of diabetic coma is the result of marked ketosis. It is related to the proportions of fat, protein, and carbohydrate oxidized in the tissues. Coma is not always correlated with high blood sugar levels; and ketosis, with relatively slight hyperglycemia, may appear in children, in elderly patients, and during infections. It can even accompany severe hypoglycemia from insulin overdosage, provided the liver glycogen is sufficiently low. Ketosis aggravates diabetic hyperglycemia and glycosuria, and it increases the resistance to insulin; recurrence of ketosis leads to permanent damage of tissues, especially the blood vessels, eyes, and liver. Diabetic coma is frequently the result of improper diet, omission of insulin, starvation, infection, vomiting, or diarrhea. The cardinal symptoms of impending coma include drowsiness, muscular weakness, nausea, flushed cheeks, abnormal respiration, dry tongue, acetone breath, acetonuria, and pains in the limbs, back, or stomach.

The liver plays a prominent role in the production of diabetic symptoms; it is largely responsible for the hyperglycemia, ketosis, excessive gluconeogenesis, lipemia, and susceptibility to infection. The diabetic liver is often enlarged, shows fatty infiltration, and has a subnormal glycogen content; pancreatectomy reduces the liver glycogen of animals to mere traces. The liver glycogen of diabetic animals is not appreciably increased by glucose administration, but the tissues can still utilize normal quantities of glucose provided the blood sugar is raised to a sufficiently high level. The oxygen uptake of minced muscle from diabetic animals or patients does not differ from the normal. Insulin deficiency is partially compensated, and many of the diabetic symptoms are alleviated, by the removal of the pituitary or the adrenals from depancreatized animals. These animals can be shown to utilize glucose, but they respond abnormally to carbohydrate intake and starvation, and they finally die in a cachectic condition. The injection of anterior pituitary extract exaggerates the

diabetic symptoms of depancreatized dogs or cats, decreases their tolerance to glucose and their sensitivity to insulin. There is a latent period of several days before these manifestations appear (page 690). The administration of adrenaline or thyroxine to normal animals causes hepatic glycogenolysis and increases the insulin requirement, but does not produce true diabetes. Injection of the corticotropic hormone causes gluconeogenesis and glycosuria in hypophysectomized, depancreatized cats, only when the adrenal glands are intact. Its effects are mediated through corticosterone and related cortical hormones.

Repeated injection of crude pituitary extract causes some degeneration of islet tissue in dogs. Primary excess of diabetogenic factor gives rise to a condition which resembles diabetes, although it is not identical with it. Less than 15 per cent of juvenile diabetic patients show pituitary involvement, manifested mostly as hypopituitary conditions, following the onset of diabetes mellitus. The urinary excretion of anterior pituitary hormones is usually normal in diabetes mellitus, but the blood and urine concentrations of chorionic gonadotropin are at times increased during diabetic pregnancy. In such instances, estrogen and progesterone administration reduces the infant mortality considerably (page 687). Diabetes is, at times, incited or aggravated by pregnancy; also, pregnant women are especially susceptible to ketosis during carbohydrate starvation. In other instances, improvement of the diabetic symptoms has been noted during late stages of pregnancy, perhaps as the result of appreciable utilization of maternal blood glucose by the fetus. Insulin does not pass the placenta; when the hormone is injected into the fetus the maternal blood sugar falls slightly, because sugar is transferred to the hypoglycemic fetus. When the injected fetus is removed, the maternal blood sugar rises promptly. Infections cause liver injury through the action of bacterial toxins, and, perhaps, by stimulating the secretion of hyperglycemic hormones. Even slight infections or colds inhibit hepatic glycogenesis, aggravate the diabetic condition, and sometimes precipitate diabetic coma.

Treatment of Diabetes Mellitus with Insulin

In well developed diabetes, hepatic carbohydrate metabolism is severely deranged; ketosis and degenerative complications ensue, and insulin must be administered to maintain health. Insulin injections compensate for the diabetic deficiency; in severe diabetes, insulin therapy must be continued. The prevention of diabetic hyperglycemia and glycosuria is a clinical ideal, not because these conditions are harmful *per se*, but because they are symptoms of a metabolic disturbance.

Since insulin is a protein, it is digested by pepsin and trypsin and is, therefore, ineffective when given by mouth. Injected insulin reduces hyperglycemia of whatever origin. In diabetes, it restores normal carbohydrate tolerance and glycogenesis in the liver and in the general tissues.

It also prevents loss of weight, and abolishes the susceptibility to infections and degenerations. The life expectancy of the properly treated diabetic patient has been increased fifteen years.

Insulin produces the most rapid and pronounced lowering of the blood sugar when it is injected intravenously. The maximal effect of subcutaneously injected insulin appears in about one hour; the hormone is usually injected one-half hour before meals, so that its maximal effect will synchronize with the peak of alimentary hyperglycemia. The effect of the injected insulin gradually decreases as the hormone is metabolized and excreted in the urine. The duration of action is proportional to the logarithm of the dose. The site of injection is varied systematically, in order to prevent tumefactions and necroses. Temporary local anaphylactoid phenomena, and permanent atrophy of the subcutaneous adipose tissue, may occur near the site of injection. The average daily dosage for diabetic patients is about 30 units of insulin; but quantities in excess of the normal daily pancreatic secretion (from 45 to 80 units) are sometimes required, inasmuch as the subcutaneously injected insulin is not delivered directly to the liver. One portion of the insulin is usually given at breakfast (to compensate the hyperglycemia of fasting), and a second portion with the evening meal. The optimal distribution of the total insulin requirement can be determined by a study of diurnal variations of hyperglycemia and glycosuria. It is often stated that 1 unit of insulin prevents the excretory loss of 2 gm. carbohydrate, but the quantity varies with the dosage and the individual, as well as with the diet, exercise, infections, ketosis, dehydration and other complications. Insulin dosage can frequently be reduced after adequate treatment is instituted, since the tissues no longer suffer from carbohydrate starvation. Certain patients require very little or no insulin after prolonged treatment. Mild or moderate exercise frequently allows reduction of the insulin dosage.

The urgent problem in diabetic coma is reduction of the ketosis by insulin injections; the glucose balance may be adjusted later. Saline injections are important to combat shock, to correct dehydration, and to prevent anuria. Sufficient insulin and carbohydrate are also important during other diabetic crises, such as operation or infection. Hemochromatosis inhibits glycogenesis, and renders both insulin and carbohydrate relatively ineffective.

The rapid temporary action of insulin has been modified by combining it with protamine and zinc salts (1 milligram zinc to 500 units insulin) to form the less soluble compounds, protamine-insulin and protamine-zinc-insulin. The latter is the more stable preparation, and it has been widely adopted to provide more prolonged and steady therapy, with fewer injections. The maximum effect develops in from twelve to twenty-four hours, as contrasted with from two to three hours for ordinary insulin. Owing to its prolonged action, excess protamine-zinc-insulin tends to produce hypoglycemic shock during the night's fast; the onset of the hypo-

glycemia is less dramatic, and is more difficultly detected than that caused by ordinary insulin. The daily dosage of protamine-zinc-insulin is about two thirds that of ordinary insulin. The latter is preferred in diabetic coma because of its quick action.

Treatment of Diabetes Mellitus by Diet

The welfare of the diabetic patient depends not only on the proper administration of insulin, but also on maintenance of optimal function through dietary regulation. The symptoms of diabetes vary with the diet. In some instances, high fat diets partially relieve hyperglycemia and glycosuria; but they decrease the effectiveness of insulin, and increase the incidence and severity of such serious complications as arteriosclerosis, cataract, ketosis, and liver degeneration. In modern practice, the diabetic patient is maintained on as liberal a carbohydrate diet as is commensurate with the insulin medication. Carbohydrate restriction is not practiced in non-diabetic hyperglycemia. High carbohydrate diets are especially beneficial in hepatic and cardiac conditions. There is no advantage in attempting to substitute other sugars for glucose, since they have no preferential physiological value in the diabetic.

The fact that high fat diets lower the blood sugar does not indicate any improvement in the fundamental diabetic condition; the phenomena are occasioned by fatty changes in the liver and by deficient hepatic function. A number of discarded insulin substitutes (synthalin, etc.) act in this fashion; they render the urine sugar free by producing hepatic insufficiency. Insulin, thiamin, and corticosterone are the only known substances which effectively improve the utilization of carbohydrate or the laboratory findings in diabetes without producing liver damage; of these, insulin is by far the most important. Thiamin often fails to relieve the neuritic symptoms of diabetes. This vitamin prevents accumulation of lactic acid and pyruvic acid in the heart, brain, and muscle tissues. The liver is less affected by thiamin deficit than are the other tissues, because it can withdraw thiamin from the portal blood.

The adjustment of the patient's caloric intake is very important, whether or not insulin be given. Fats must be kept well below the ketogenic ratio (page 227). The caloric intake must be adequate for the maintenance of an approximately normal body weight, in order to prevent gluconeogenesis of body protein and malignant cachexia. The daily diet should include from 1.0 to 1.5 gm. protein per kg. of body weight. The "high" carbohydrate diabetic diet usually contains only from 100 to 200 gm. carbohydrate daily, as compared with 400 gm. for the average normal man. However, this quantity is still considerably above the ketogenic level.

It is important to distribute the daily caloric intake in accordance with the needs of the individual patient. Routine tests for glycosuria at inter-

vals during the day assist in determining the proper distribution of food, and the time for insulin medication. Diabetics are usually given test diets whose caloric value, and carbohydrate and protein contents, are known; by repeated urinary sugar tests, and modification of the test diet, the tolerance of the patient is determined. The optimal tolerance can be determined only after the alleviation of ketosis and other complications which reduce the carbohydrate tolerance. Details of the quantitative dietary control of diabetes are beyond the scope of this book; they may be found in the clinical and dietetic references.

Too rapid adjustment of severe diabetes by diet and insulin may cause edema and visual disturbance. Hypoglycemia is avoided in cardiac cases, since lowering of the glucose supply to the heart may cause cardiac failure. Emotional disturbance at meal time may delay absorption of carbohydrate and lead to hypoglycemia from the administered insulin. The education of the patient is a very important adjunct to diabetic therapy; such education is best begun in the hospital, especially in the case of children.

Sodium chloride administration is useful in treating dehydration and abdominal pain accompanying ketosis.

HYPERGLYCORRACHIA AND HYPOGLYCORRACHIA

The glucose content of the cerebrospinal fluid varies with the blood sugar level, the permeability of the choroid plexus and the cerebrospinal capillaries, and with the cell count of the cerebrospinal fluid. After a night's fast, the reducing sugar level of the cerebrospinal fluid is normally 55 ± 15 mg. per cent in adults, and 80 ± 10 mg. per cent in children. For clinical evaluation, the sugar content of the cerebrospinal fluid must be compared with the blood sugar level. Increased cerebrospinal fluid sugar, or hyperglycorrachia, occurs in hyperglycemic conditions, in acute epidemic encephalitis, when the intracranial tension is increased (as in convulsions, brain tumors, or abscesses), and in some cases of dementia praecox, uremia, and syphilis. In syphilitic tabes and paresis, the level is usually normal, while in acute meningeal involvement it is slightly low. Hypoglycorrachia occurs when the cell count (bacteria, leukocytes, erythrocytes) is increased, as in acute meningitis of meningococcal or tubercular origin. Glycolysis by the cellular components lowers the cerebrospinal fluid sugar, hence the spinal fluid samples must be analyzed promptly.

BIBLIOGRAPHY

CHEMISTRY

General

ARMSTRONG, E. F., and ARMSTRONG, K. F. *The Carbohydrates*. Ed. 5. New York, Longmans, Green, 1934.

- BROWNE, C. A., and ZERBAN, F. W. *Physical and Chemical Methods of Sugar Analysis*. New York, Wiley, 1941.
- DEGERING, E. F. *Outline of the Chemistry of the Carbohydrates*. Lafayette, Purdue Univ. Press, 1941.
- EVERETT, M. R., and SHEPPARD, F. *Oxidation of Carbohydrates in Acid Solution*. Oklahoma City, Univ. of Oklahoma School of Medicine, 1936. *Keturonic Acids, Salt Catalysis*. Oklahoma City, Univ. of Oklahoma School of Medicine, 1944.
- GILMAN, H. *Organic Chemistry*. Ed. 2. Vol. II. New York, Wiley, 1943.
- MICHEEL, F. *Chemistry of the Sugars and the Polysaccharides*. New York, Interscience Pub., 1945.
- PIGMAN, W. W., and WOLFROM, M. L. *Advances in Carbohydrate Chemistry*. New York, Academic Press, 1944.

Stereoisomerism; Optical Activity

- GILMAN, H. *Organic Chemistry*. Ed. 2. Vol. I. New York, Wiley, 1943.
- KAUZMAN, W. J., *et al.* Theories of optical rotatory power. *Chem. Rev.*, 26 : 339, 1940.

Glycosides; Individual Sugars

- GILMAN, H. *Organic Chemistry*. Ed. 2. Vol. II. New York, Wiley, 1943.
- KING, C. G. Chemistry of vitamin C. *J. A. M. A.*, 111 : 1462, 1938.
- LAWRENCE, W. J. C., and PRICE, J. R. Genetics and chemistry of flower colors. *Biol. Rev. Cambridge Phil. Soc.*, 15 : 35, 1940.
- LEVENE, P. A. T. *Hexosamines and Mucoproteins*. New York, Longmans, Green, 1925.
- MAYER, F. *The Chemistry of Natural Coloring Matters*. New York, Reinhold, 1943.
- RIJN, J. J. L. VAN. *Die Glykoside*. Ed. 2. Berlin, Gebrüder Borntraeger, 1931.

Polysaccharides

- HINTON, C. L. *Fruit Pectins*. New York, Chemical Pub. Co., 1940.
- MEYER, K. H. *Natural and Synthetic High Polymers*. New York, Interscience Pub., 1942.
- MEYER, K. H. Recent developments in starch chemistry. *Adv. in Colloid Sc.*, 1 : 143, 1942.
- MEYER, K. H. The chemistry of glycogen. *Adv. in Enzymol.*, 3 : 109, 1943.
- NORMAN, A. G. *Biochemistry of Cellulose, Polyuronides, Lignin, Etc.* London, Oxford Univ. Press, 1937.
- OTT, E., *et al.* *Cellulose and Cellulose Derivatives*. New York, Interscience Pub., 1943.
- RADLEY, J. A. *Starch and Its Derivatives*. Ed. 2. New York, D. Van Nostrand, 1944.

METABOLISM

General

- DEUEL, H. J., JR. Metabolism of fructose and galactose. *Physiol. Rev.*, 16 : 173, 1936.

Photosynthesis

- RABINOWITCH, E. I. *Photosynthesis*. New York, Interscience Pub., 1945.
VAN NIEL, C. B. The bacterial photosyntheses. *Adv. in Enzymol.*, 1 : 263, 1941.
WERKMAN, C. H., and WOOD, H. G. Heterotrophic assimilation of carbon dioxide. *Adv. in Enzymol.*, 2 : 135, 1942.

Digestion; Absorption; Fermentation

- LEIBOWITZ, J., and HESTRIN, S. Alcoholic fermentation of the oligosaccharides. *Adv. in Enzymol.*, 5 : 87, 1945.
NORD, F. F. Mechanism of alcoholic fermentation. *Chem. Rev.*, 26 : 423, 1940.
PIGMAN, W. W. The glycosidases. *Adv. in Enzymol.*, 4 : 41, 1944.
VERZAR, F., and McDougall, E. J. Absorption from the Intestine. New York, Longmans, Green, 1936.
WERKMAN, C. H., and WOOD, H. G. Metabolism of bacteria. *Botan. Rev.*, 8 : 1, 1942.

Blood and Tissue Sugar; Glycogen

- SOSKIN, S. The blood sugar. *Physiol. Rev.*, 21 : 140, 1941.
SOSKIN, S. Storage and significance of tissue glycogen in health and disease. *Arch. Int. Med.*, 71 : 219, 1943.

Glycolysis; Oxidation; Phosphorylation

- BARRON, E. S. G. Mechanisms of carbohydrate metabolism. *Adv. in Enzymol.*, 3 : 149, 1943.
CORI, C. F. Phosphorylation of glycogen and glucose. *Biol. Symposia*, 5 : 131, 1941.
DORFMAN, A. Pathways of glycolysis. *Physiol. Rev.*, 23 : 124, 1943.
EVANS, E. A., JR. The fixation of carbon dioxide by animal tissues. *Harvey Lect.*, 39 : 273, 1943-1944.
KALCKAR, H. M. The function of phosphate in enzymic syntheses. *Ann. New York Acad. Sc.*, 45 : 395, 1944.
KREBS, H. A. Intermediary stages in the biological oxidation of carbohydrate. *Adv. in Enzymol.*, 3 : 191, 1943.
LIPMANN, F. Metabolic generation and utilization of phosphate bond energy. *Adv. in Enzymol.*, 1 : 99, 1941.
MEYERHOF, O. Energy relation in glucolysis and phosphorylation. *Ann. New York Acad. Sc.*, 45 : 377, 1944.
STOTZ, E. Pyruvate metabolism. *Adv. in Enzymol.*, 5 : 129, 1945.

Muscle and Nerve Metabolism

- CRUICKSHANK, E. W. H. Cardiac metabolism. *Physiol. Rev.*, 16 : 597, 1936.
FENN, W. O. Muscle. *Biol. Symposia*, Vol. III. Lancaster, Cattell Press, 1941.
FISCHER, E. Vertebrate smooth muscle. *Physiol. Rev.*, 24 : 467, 1944.
GEMMILL, C. L. The fuel for muscular exercise. *Physiol. Rev.*, 22 : 32, 1942.
PAGE, I. H. *Chemistry of the Brain*. Springfield, Thomas, 1937.

- WORTS, W. Some nutritional aspects of brain metabolism. *Psychiatric Quart.*, 15 : 693, 1941.

Effects of Hormones

- HAIST, R. E. Factors affecting the insulin content of the pancreas. *Physiol. Rev.*, 24 : 409, 1944.
- JENSEN, H. F. *Insulin, Its Chemistry and Physiology*. New York, Commonwealth Fund, 1938.
- LONG, C. N. H., *et al.* The adrenal cortex and carbohydrate metabolism. *Endocrinology*, 26 : 309, 1940.
- SOSKIN, S. Role of the endocrines in the regulation of blood sugar. *J. Clin. Endocrinol.*, 4 : 75, 1944.
- WATERS, E. T., and BEST, C. H. The pancreas as an organ of internal secretion. *J. A. M. A.*, 117 : 852, 1941.
- YOUNG, F. G. The pituitary gland and carbohydrate metabolism. *Endocrinology*, 26 : 345, 1940.

Miscellaneous

- CARPENTER, T. M. The metabolism of alcohol. *Quart. J. Stud. on Alcohol*, 1 : 201, 1940.
- HALLMAN, N. The formation and destruction of citric acid in animal tissues. *Acta Physiol. Scandinav.*, Suppl. 5, 1940.
- McKEE, F. W., and HAWKINS, H. B. Phlorhizin glucosuria. *Physiol. Rev.*, 25 : 255, 1945.
- NEEDHAM, J. *Biochemistry and Morphogenesis*. New York, Macmillan, 1942.
- SMITH, A. H., and ORTEN, J. M. Nutritional and metabolic significance of certain organic acids. *J. Nutrition*, 13 : 601, 1937.

PATHOLOGY

Diabetes Mellitus

- JOSLIN, E. P., *et al.* *Treatment of Diabetes Mellitus*. Ed. 7. Philadelphia, Lea and Febiger, 1940.
- LONG, C. N. H. Endocrine control of carbohydrate metabolism and its relation to diabetes in man. *Proc. Am. Diabetes Assoc.*, 2 : 97, 1942.
- STADIE, W. C. Intermediate metabolism in diabetes mellitus. *Harvey Lect.*, 37 : 129, 1941-42.
- WILDER, R. M. *Clinical Diabetes Mellitus and Hyperinsulinism*. Philadelphia, Saunders, 1940.

Miscellaneous

- BOCK, J. C. The benign melliturias. *Physiol. Rev.*, 24 : 169, 1944.
- CHAMBERS, W. H. Undernutrition and carbohydrate metabolism. *Physiol. Rev.*, 18 : 248, 1938.
- CREVELD, S. VAN. Glycogen disease. *Medicine*, 18 : 1, 1939.
- HIMWICH, H. E. Hypoglycemia, its physiology, pathology, symptomatology and treatment. *Am. J. Digest. Dis. & Nutrition*, 11 : 1, 1944.
- KATZENELBOGEN, S. Shock therapies in the major psychoses. *Psychiatry*, 3 : 409, 1940.

- MARTIN, E. *Dextrose Therapy in Everyday Practice*. New York, Hoeber, 1937.
- MARTIN, W., *et al.* Insulin resistance. *J. Clin. Endocrinol.*, 1 : 387, 1941.
- McFARLAND, R. A., and GOLDSTEIN, H. The biochemistry of dementia precox. *Am. J. Psychiat.*, 95 : 509, 1938.
- SACHS, B., *et al.* Essential fructosuria. *Am. J. Dis. Child.*, 63 : 252, 1942.
- WILDER, R. M. Therapy with preparations of pancreas. *J. A. M. A.*, 117 : 930, 1941.

Cerebrospinal Fluid Sugar

- KATZENELBOGEN, S. *The Cerebrospinal Fluid and Its Relation to the Blood*. Baltimore, Johns Hopkins Press, 1935.
- MERRITT, H. H., and FREMONT-SMITH, F. *The Cerebrospinal Fluid*. Philadelphia, Saunders, 1937.

CHAPTER VI

PROTEINS



CHEMISTRY

"Scientific reflection always assumes a larger world than that which is immediately before us." — MORRIS R. COHEN

CLASSIFICATION

Proteins are complex nitrogenous compounds of α -amino acids. They are characteristic components of protoplasm and have highly specific physiological activities. The structure and chemical composition of proteins determine both the morphological and the metabolic aspects of living organisms.

A chemical classification of proteins is highly desirable but not practicable at present. The temporary classification given in Tables 64 and 70 (page 389) is based largely on comparative solubilities and other physical properties. Table 64 presents a summary of the protein decomposition products or *derived proteins* with which our study begins.

TYPES AND DISTRIBUTION OF AMINO ACIDS

The fundamental units of proteins are α -amino acids, classified in Table 65 as aliphatic, cyclic, and sulfur-containing amino acids and their branched chain, hydroxy and iodo derivatives. The cyclic amino acids have either pyrrolidine, imidazole, indole, or benzene nuclei. The majority of amino acids may be regarded as derivatives of alanine.

The distribution of amino acid units varies greatly in different proteins (Table 66, page 355). Silk fibroin, collagens, gelatins, protamines, and the proteins of cereal seeds are deficient in several amino acids; these, and the storage proteins of nuts, lack several of the acids which are dietary essentials for mammals (indicated by *E* in Table 65). Hemoglobin has a low isoleucine content. Protamines contain a very limited assortment of amino acid units; salmin has only arginine, proline, serine, and valine; and sturin contains only arginine, histidine, and lysine. Protamines are the only sulfur-free proteins. In the average protein, glutamic acid and leucine occur in larger quantities than do the other individual amino acids. Some

TABLE 64

CLASSIFICATION OF PROTEINS, I

I. Derived proteins (decomposition products of proteins).	
<i>Hydrolytic products</i> (in order of increasing molecular size).	
<i>Amino acids</i>	Fundamental chemical units of proteins; soluble in water; dialyzable; negative biuret test.
<i>Peptides</i>	Soluble in water; dialyzable.
<i>Simple peptides</i>	Short chains of amino acid units; negative or pink biuret test. <i>Examples:</i> carnosine, glutathione, protons.
<i>Polypeptides</i>	Longer chains of amino acid units; pink biuret test. <i>Examples:</i> gramicidin, leukotaxine, pitocin, pitressin, secretin, tyrocidine.
<i>Peptones</i>	Soluble in water; dialyzable; flocculated by phosphotungstic, tannic, or trichloracetic acid; pink biuret test. <i>Examples:</i> fibrin peptone. Bacto-peptone is a commercial source.
<i>Proteoses (or albumoses)</i>	Soluble in water; pink biuret test. <i>Examples:</i> albumose, Bence-Jones protein, caseose, gelatose, globulose, hirudin, hypertensin, rennin. Bactoprotone and Witte's peptone (from fibrin) are commercial sources.
<i>Secondary or deutero-proteoses</i>	Flocculated by saturation with ammonium sulfate.
<i>Primary or proto-proteoses</i>	Flocculated by half saturation with ammonium sulfate; flocculated by ferrocyanic, nitric, phosphotungstic, picric, tannic, or trichloracetic acid, or by sodium chloride plus hydrochloric acid at room temperature, with resolution of the flocculate upon heating.
<i>Metaproteins</i> ¹	Insoluble in water or neutral solutions; soluble in weak acids or alkalis; flocculated by half saturation with ammonium sulfate; coagulated by heat. <i>Examples:</i> acid albuminate, alkali albuminate (Lieberkühn's jelly), paracasein, syntonin (acid myosin).
<i>Proteans</i> ¹	Insoluble initial digestion products. <i>Examples:</i> edestan, fibrin, myogen fibrin, myosan (myosin fibrin).
<i>Denatured proteins</i> ¹	Proteins chemically altered by acid, alkali, electrolytes, heat, organic solvents, pressure, irradiation, or shaking; insoluble at their isoelectric points. <i>Examples:</i> coagulated albumin, coagulated globulin.
II. Simple proteins ¹	Complex compounds which contain chiefly amino acid units, or their derivatives. (See Table 70, page 389, for classification.)
III. Conjugated proteins ¹	Complex compounds which contain both amino acid units and prosthetic units. (See Table 70, page 391, for classification.)

¹ Metaproteins, proteans, and proteins give the ordinary violet to purple biuret test.

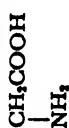
cereal proteins yield as much as 43 per cent glutamic acid and considerable proline. Histidine, hydroxylysine, methionine, threonine, and tryptophane are usually minor constituents of proteins, and the three last named do not appear in any protein in large quantity. The protamines

TABLE 65
AMINO ACIDS
(The Structural Units of Proteins)

I. ALIPHATIC SERIES

Monocarboxylic Acids

Glycine
(α -Aminoacetic Acid)



d-Alanine
(α -Aminopropionic Acid)

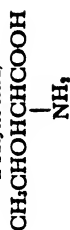


(Hydroxy Acids)

L-Serine
(α -Amino- β -hydroxy-
propionic Acid)

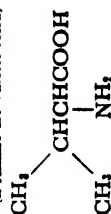


d-Threonine (E)
(α -Amino- β -hydroxy-
n-butyric Acid)

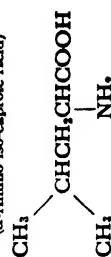


(Branched Chain
Acids)

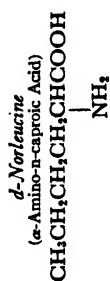
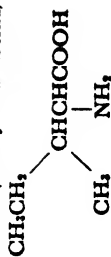
d-Valine (E)
(α -Amino-iso-valeric Acid)



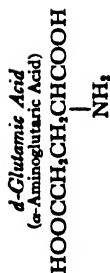
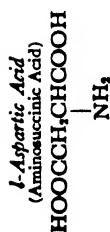
L-Leucine (E)
(α -Amino-iso-caproic Acid)



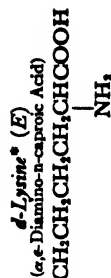
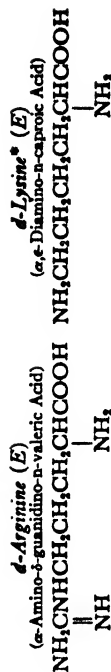
d-Isoleucine (E)
(α -Amino- β -methyl-n-valeric Acid)



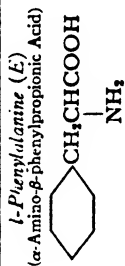
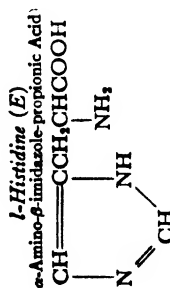
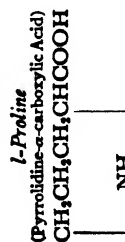
Dicarboxylic Acids



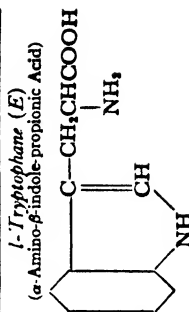
Diamino Acids



II. CYCLIC SERIES



Aromatic



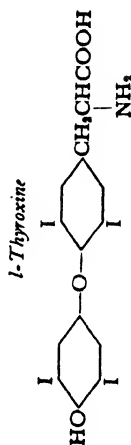
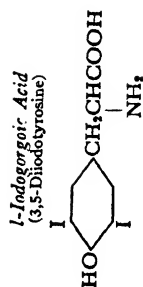
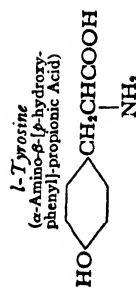
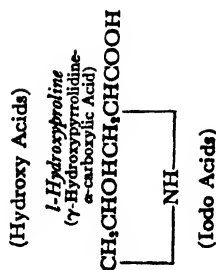
* Hydroxylysine has been isolated from protein, but its constitution is undetermined.

TABLE 65 (Cont.)

AMINO ACIDS

(The Structural Units of Proteins)

II. CYCLIC SERIES



III. SULFUR-CONTAINING SERIES

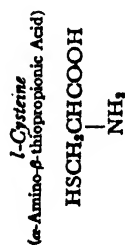


TABLE 66

DISTRIBUTION OF AMINO ACIDS IN PROTEINS ^{1,2}

<i>Amino acid</i>	PROTEINS WHICH CONTAIN LARGE QUANTITIES		PROTEINS WHICH CONTAIN LESS THAN 1 PER CENT	AVERAGE IN REMAINING PROTEINS
		<i>Per Cent</i>		<i>Per Cent</i>
Alanine	Gramicidin	30	Salmin, vitellin	4 (1-10)
	Fibroin ³	26.5		
	Tyrocidine	13.5		
Arginine	Clupein	88	Elastin, fibroin, pitocin	6.5 (2-17)
	Salmin	87.5		
	Histone ⁴	27		
Aspartic acid	Fibrin	12	Clupein, elastin, salmin	4.5 (1-9)
	Edestin	10		
	β -Lactoglobulin	10		
	Tyrocidine	10		
	Serum albumin	10		
	Lactalbumin	9.5		
	Glycinin	9.5		
Cystine	Pitressin	19	Casein, clupein, elastin,	2 (1-6)
	Keratins	1-19	gelatin, hemoglobin, my-	
	Pitocin	18.5	osin, salmin, secretin,	
	Insulin	12.5	tobacco mosaic virus,	
			yellow enzyme, zein	
Glutamic acid	Gliadin	43.5	Clupein, histone, salmin	15 (2-26)
	Hordein	41.5		
	Zein	35.5		
	Insulin	30		
Glycine	Fibroin	44	Albumins, amandin, casein,	4 (1-13)
	Elastin	29.5	clupein, excelsin, gliadin,	
	Gelatin ⁵	27	glutenin, histone, hemo-	
			globin, hordein, legumin,	
			salmin, tobacco mosaic	
			virus, zein	
Histidine	Insulin	10.5	Clupein, elastin, ferritin,	1.5 (1-5)
	Hemoglobin	7.5	fibroin, keratins, pepsin,	
	Hemocyanin	6.0	ricin, salmin, tobacco	
			mosaic virus, zein	
Hydroxylysine	Collagen	0.9	Arachin, casein, clupein,	
	Gelatin	0.9	gliadin, hemoglobin, ker-	
			atins, lactalbumin, β -lac-	
			toglobulin, ovalbumin,	
			papain, salmin, zein ⁶	
Hydroxyproline	Gelatin	13.5	Bence-Jones protein, ca-	
			sein, clupein, gramicidin,	
			hemoglobin, ricin, sal-	
			min, serum albumin, se-	
			rum globulin, yellow en-	
			zyme, zein	
Leucine + isoleucine	Elastin	30	Clupein, salmin	10.5 (2-22)
	Insulin	30		
	Gramicidin	30		
	Zein	27.5		
	β -Lactoglobulin	24		

TABLE 66 (Cont.)

DISTRIBUTION OF AMINO ACIDS IN PROTEINS^{1,2}

	PROTEINS WHICH CONTAIN LARGE QUANTITIES		PROTEINS WHICH CONTAIN LESS THAN 1 PER CENT	AVERAGE IN REMAINING PROTEINS
<i>Amino Acid</i>		<i>Per Cent</i>		<i>Per Cent</i>
Lysine	Cytochrome <i>c</i>	24.5	Amandin, clupein, fibroin, gliadin, hordein, salmin, tobacco mosaic virus, zein	4 (1-9)
	Yellow enzyme	13.5		
	β -Lactoglobulin	10		
	Serum albumin	10		
	Hepatoalbumin	10		
	Myosin	10		
	Histone	9.5		
Methionine	Ovalbumin	5	Arachin, Bence-Jones pro- tein, clupein, elastin, gelatin, insulin, keratins, salmin, serum albumin, tobacco mosaic virus	2.5 (1-4)
Phenylalanine	Tyrocidine	22	Clupein, gramicidin, salmin	3.5 (1-8)
Proline	Fibroin	11.5		
	Gelatin	17	Gramicidin, myosin	4.5 (1-9)
	Elastin	15		
	Collagen	15		
	Gliadin	12		
Serine	Salmin	11	Edestin, excelsin, gliadin, glutenin	3.5 (1-6)
	Fibroin	13.5		
	Serum γ -glob- ulin	11.5		
	Fibrin	10		
	Vitellin	9		
Threonine	Salmin	8	Clupein, salmin, tyrocidine	3 (1-6)
	Ovalbumin	7.5		
	Chymotryp- sinogen	9.5		
	Pepsin	9.5		
	Serum γ -glob- ulin	8.5		
	Fibrin	6.5		
	Tobacco mosaic virus	6.5		
Tryptophane	Gramicidin	36	Arachin, clupein, conara- chin, elastin, gelatin, gli- adin, histones, hordein, insulin, myosin, ricin, salmin, secretin, serum albumin, zein	1.5 (1-4)
	Hemocyanin	5.5		
	Chymotryp- sinogen	5.5		
	Conalbumin	5		
	Yellow enzyme	5		
	Trypsin	4.5		
	Tobacco mosaic virus	4.5		
Tyrosine	Pitocin	14	Clupein, gelatin, gramici- din, salmin, secretin	4 (1-8)
	Tyrocidine	13.5		
	Fibroin	13		
	Insulin	12.5		
	Pitressin	12		
	Pepsin	10.5		

TABLE 66 (Cont.)

DISTRIBUTION OF AMINO ACIDS IN PROTEINS ^{1,2}

Amino Acid	PROTEINS WHICH CONTAIN LARGE QUANTITIES		PROTEINS WHICH CONTAIN LESS THAN 1 PER CENT	AVERAGE IN REMAINING PROTEINS
		Per Cent		Per Cent
Valine	Gramicidin	22	Amandin, glutenin, hordein	3 (1-6)
	Elastin	13.5		
	Tyrosidine	10		
	Hemoglobin	9		
	Casein	8		
	β -Lactoglobulin	7.5		
Amide nitrogen (as NH ₃)	Hepatoglobulin	12	Clupein, elastin, gelatin, salmin	3 (1-5)
	Pepsin	9		
	Hepatoalbumin	8		
	Histone	7.5		

¹ Partial data, obtained from 60 representative proteins.

² The complete yield of amino acids should be approximately 115 per cent, because of the addition of water during hydrolysis.

³ The fibroin indicated is from silk.

⁴ The histone indicated is from the thymus gland.

⁵ Gelatin and collagen have similar compositions.

⁶ These proteins contain less than 0.3 per cent hydroxylysine.

and histones contain large proportions of the basic amino acids (arginine, histidine, and lysine); thus, salmin and clupein have about 88 per cent arginine. Cytochrome *c*, another basic protein, contains approximately 25 per cent lysine. Unusually large quantities of alanine, glycine, phenylalanine, serine, and tyrosine occur in fibroin, while collagen and gelatin contain considerable glycine, hydroxyproline, and proline. Insulin and the keratins have the largest quantities of cystine (plus cysteine); insulin also has exceptionally large amounts of histidine, leucine, and tyrosine. The determined amino acid composition of a protein depends to some extent on the technique of isolation. β -Hydroxyglutamic acid is no longer regarded as a preformed amino acid unit of native proteins.

Optical Activity

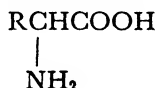
With the exception of glycine, the α -amino acids are optically active; the natural isomers listed in Table 65 have configurations related to *l*-lactic acid and the fifth carbon atom of *l*-glucose. The ordinary nomenclature, as given in the table, indicates the direction of rotation of polarized light except in the cases of aspartic acid, cysteine, and threonine. At its α -carbon atom, *d*-threonine has the configuration of *l*-lactic acid; but threonine was named from its relation to the sugar, *d*-threose. Cystine, hydroxyproline, isoleucine, and threonine have two asymmetric carbon atoms and four stereoisomeric forms. Amino acid enantiomorphs exhibit

different physiological behaviors; the *l*-forms occur as protein units and are actively metabolized by living tissues. The unnatural *d*-forms have generally a sweet taste, and they are metabolized more slowly than the *l*-forms. The *d*-amino acids occur only in traces in ordinary proteins, but they are important constituents of anthrax and *B. mesentericus* polypeptides, gramicidin and tyrocidine, and ergot alkaloids.

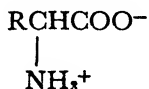
The rotations of the anions and cations of amino acids and proteins differ from the rotations of the free or isoelectric molecules. Optically active amino acid units of peptides and proteins are racemized rapidly by alkali, but only very slowly by acids. Cystine can be racemized by heating in strongly acid solution; but other free amino acids and those amino acid units of proteins and peptides which have free carboxyl radicals are not racemized by acid or alkali. Acetylated amino acids can be racemized by treatment with acetic anhydride.

Amino Acids as Ampholytes

The general formula for α -amino acids,



indicates that the characteristic reactive radicals are the basic NH_2 and the acidic COOH . Each *basic amino acid* (arginine, histidine or lysine) has a second basic nitrogenous radical, and each *dicarboxylic acid* (aspartic, or glutamic acid) has a second carboxyl radical. Amino acids cannot be titrated effectively by ordinary methods owing to the gradual change in pH at the endpoint. In Figure 8 the titration curves of monoamino, basic, and dicarboxylic amino acids are compared. The intersections of the curves with the zero abscissa approximately locate the isoelectric points (pH 3.2, 6.1, and 10.8 for glutamic acid, glycine, and arginine, respectively). Isoelectric points for other amino acids are given in Table 10, page 47. At their isoelectric points amino acids exist as electrically neutral, doubly charged amphions, dipolar ions, or zwitter ions,



Under the influence of the electric current such amphions migrate neither to the anode nor to the cathode. The isoelectric point of an amino acid, protein, or related substance may be defined as the pH at which the substance carries maximal zwitter ion charges. An aqueous solution of a pure amino acid or protein is not exactly at its isoelectric point.

Amino acids can be titrated in the presence of formaldehyde to a sharp

endpoint at pH 9 to 10, with phenolphthalein as indicator. In this *formol* or *Sørensen titration*, formaldehyde apparently increases the ionization of hydrogen from the zwitter ions as follows:

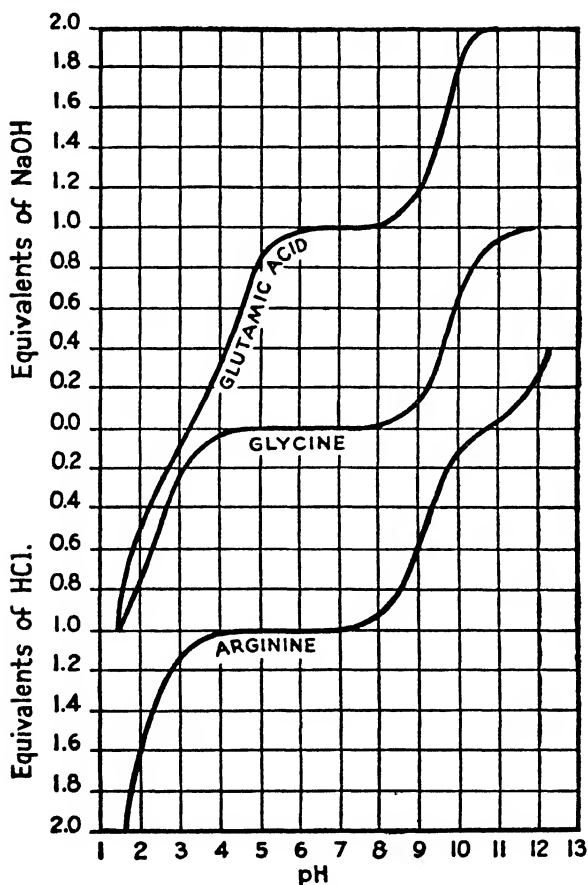
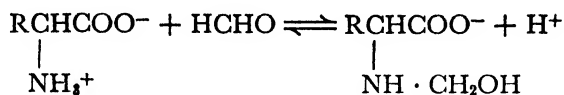
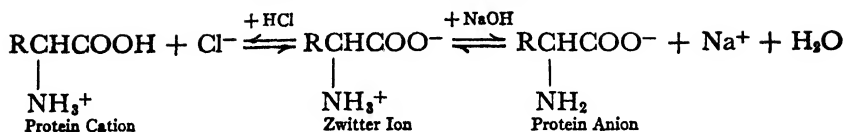


FIG. 8. Titration curves of amino acids.

Approximately 7.5 per cent neutral formaldehyde should be present for quantitative titration. Ammonia, magnesium, and phosphates interfere with the method. The formol titration has been used extensively to determine the rate of liberation of amino acids during protein digestion.

When amino acids and proteins react with acids or bases, the dipolar ions form salts as follows:



In sufficiently acid solutions amino acids and proteins become charged positively, while in alkaline solutions they have negative charges.

With the exception of tyrosine and cystine, the amino acids are rather soluble in water. The pyrrolidine acids dissolve in anhydrous alcohol. All amino acids are soluble in ordinary dilute acids and alkalis. With certain acids they form insoluble salts that are useful for isolation. Nitranilic acid is used as an amino acid precipitant; flavianic acid forms insoluble arginine diflavanate; picric acid gives well crystallized arginine, glycine, histidine, lysine, and proline picrates; and picrolonic acid forms rather insoluble crystalline picrolonates from which the amino acids may be regenerated by acidifying and extracting the picrolonic acid with ether. The so-called alkaloidal reagents (phosphomolybdic acid, phosphotungstic acid, etc.) precipitate basic amino acids selectively. In alkaline solution, carbon dioxide unites with the amino radicals of amino acids and proteins to form carbamino derivatives. (See carbohemoglobin, page 24.)

Amino acids also form difficultly soluble crystalline salts with such metallic cations as barium, calcium, copper, gold, lead, mercury, platinum, silver, and zinc. The barium and calcium salts of aspartic and glutamic acids are precipitated quantitatively by alcohol. The silver compounds of arginine and histidine are insoluble in alkaline solutions. Mercuric sulfate dissolved in 5 per cent sulfuric acid precipitates cystine, histidine, and tryptophane quantitatively.

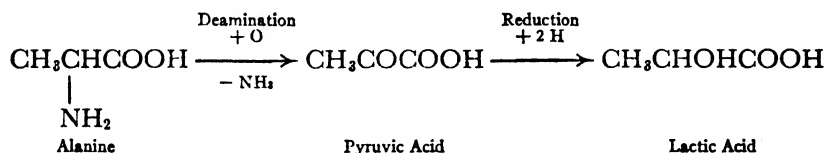
Alkali cations unite in true salt linkage with amino acid anions, but polyvalent and metallic cations tend to form double salts and coordination complexes with the zwitter ions of amino acids and proteins. The steric configurations of amino acids influence the formation of such complexes. Glycine is precipitated selectively by potassium trioxalato-chromate or cobaltate, and it may be determined quantitatively by these reagents. The complexes formed by Reinecke salt, $[(\text{NH}_3)_2\text{Cr}(\text{CNS})_4]\text{NH}_4$, are useful for separating and isolating amino acids. The amino acids are precipitated as complexes from alkaline solution by mercuric acetate, but this precipitation is not quantitative for all amino acids.

Other methods of separating amino acids include the original Fischer process of esterification and fractional distillation of the amino acid esters *in vacuo*. Here, considerable loss occurs and the amino acids are partially racemized. Such complications are avoided by Dakin's method of continuous extraction of the amino acid syrup *in vacuo* with butyl alcohol. The monoamino acids pass into the butyl alcohol extract, while the basic and dicarboxylic acids remain in the aqueous phase. Another method of separating amino acids is electrical transport in a three compartment cell.

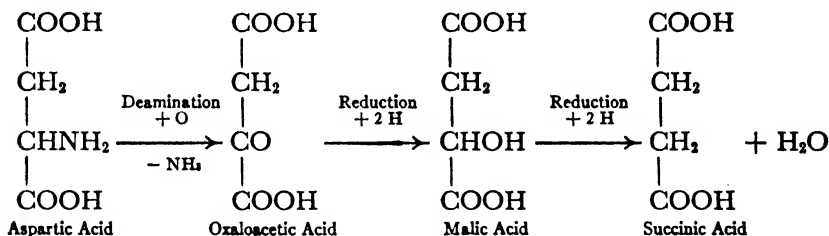
At pH 5.5 dicarboxylic acids migrate to the anode and basic acids to the cathode. Histidine can be separated by re-electrolyzing the basic fraction at pH 7.5.

Deamination

Oxidative deamination of amino acids is an important biological reaction. Oxidation of α -amino acids by hydrogen peroxide or permanganate transforms them into α -keto acids. In biological systems, the keto acids can be reduced to the corresponding hydroxy acids:

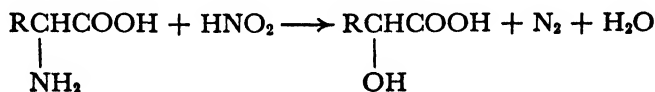


Biological reduction (as in strict bacterial anaerobiosis) can proceed to the formation of fatty acids, as, for example, acetic acid from glycine, propionic acid from alanine, isovaleric acid from leucine, indolepropionic acid from tryptophane, and succinic acid from aspartic acid:



The formulæ and derivation of important deamination products of amino acids are given in Table 67. Those from alanine, arginine, cystine, dicarboxylic acids, glycine, histidine, methionine, proline, serine, threonine, and valine are gluconeogenic (Table 40, page 226), while the deaminated acids from isoleucine, leucine, phenylalanine, and tyrosine are ketogenic.

Deamination of amino acids by nitrous acid is the basis of Van Slyke's method for determining free amino radicals:

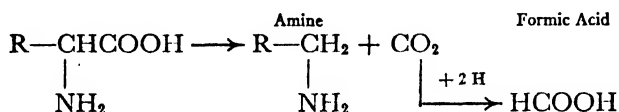


The α -amino nitrogen is liberated quantitatively and can be measured in a special apparatus. Cystine and glycine give somewhat high values in this method, and a portion of the ϵ -amino radical of lysine and the acid amide nitrogen of glutamine is also liberated by nitrous acid. The pyrrolidine

acids do not react with nitrous acid but aminopurines, creatinine, and urea do. Despite these complications, the Van Slyke method has proved very valuable for determining small quantities of amino acids and the free amino radicals of proteins. Alanine can be determined quantitatively as lactic acid, after deamination by nitrous acid. Deamination of certain enzymes, hormones, and toxins by nitrous acid reduces their physiological activity.

Decarboxylation

Bacteria and fungi can decarboxylate amino acids, and produce amines:



Increasing acidity accelerates bacterial decarboxylation; under anaerobic conditions the carbon dioxide is, at times, reduced to formic acid. The amines and phenols produced by the catabolism of amino acids are termed *aporrhegas* (Table 67). Some of these amines are sympathomimetic and increase blood pressure; the aromatic amines are most effective. Tyramine and phenylethylamine have, respectively, 0.05 and 0.0125 the pressor activity of adrenaline. Histamine, which is present in small quantities in many tissues, causes powerful vasodilatation, lowers blood pressure, increases capillary permeability, stimulates smooth muscle directly, produces uterine contractions, and, when injected intracutaneously, causes itching or urticaria. It has a gastric secretagogue action, and is tentatively identified as the anaphylatoxin (pages 138 and 488).

Cadaverine and putrescine are *diamines*. They have little physiological activity. Spermine and spermidine have the following formulae:

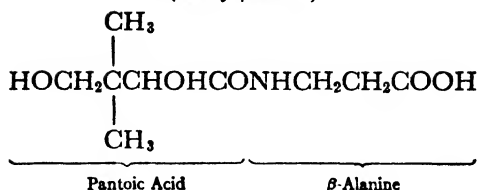


Spermine is found in numerous tissues in concentrations of from 1 to 30 mg. per cent (as phosphate), and in larger quantities in the prostate and in its secretion. Human semen contains from 90 to 200 mg. per cent, and the human prostate 130 mg. per cent of spermine phosphate. Crystals of the salt have been termed Charcot's crystals; other crystals described under this name are probably of protein nature. The prostate has considerable phosphatase, which may be concerned in the metabolism of the diamines. The odor of semen is traceable to spermidine.

β -Alanine is a growth accessory for micro-organisms; it is also a constituent of *l*-carnosine (a depressor substance found in muscles), and of the vitamin, pantothenic acid. In the latter, it is in peptide linkage with pantoic acid:

PANTOTHENIC ACID

(Pantoyl- β -alanine)



Indole and skatole are discussed on page 378.

TABLE 67

IMPORTANT BIOLOGICAL DEAMINATION AND
DECARBOXYLATION PRODUCTS OF AMINO ACIDS

AMINO ACID	DEAMINATION PRODUCTS	DECARBOXYLATION PRODUCTS (APORRHEGMAS)
<i>Alanine</i> CH_3CHCOOH $ $ NH_2	<i>Pyruvic Acid</i> $\text{CH}_3\text{COCOCH}_3$	<i>Ethylamine</i> $\text{CH}_3\text{CH}_2\text{NH}_2$
	<i>Lactic Acid</i> $\text{CH}_3\text{CHOHCOOH}$	
<i>Arginine</i> $\text{NH}_2\text{CNHCH}_2\text{CH}_2\text{CH}_2\text{CHCOOH}$ $\parallel \qquad \qquad $ $\text{NH} \qquad \qquad \text{NH}_2$	<i>Citrulline</i> $\text{NH}_2\text{CONHCH}_2\text{CH}_2\text{CH}_2\text{CHCOOH}$ $\qquad \qquad \qquad $ $\qquad \qquad \qquad \text{NH}_2$	<i>Agmatine</i> $\text{NH}_2\text{CNHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ \parallel NH
<i>Aspartic Acid</i> $\text{HOOCCH}_2\text{CHCOOH}$ $ $ NH_2	<i>Oxaloacetic Acid</i> $\text{HOOCCH}_2\text{COCOCH}_3$	<i>β-Alanine</i> $\text{HOOCCH}_2\text{CH}_2\text{NH}_2$
	<i>Malic Acid</i> $\text{HOOCCH}_2\text{CHOHCOOH}$	
<i>3,4-Dihydroxyphenylalanine</i> HO $\text{HO}-\text{C}_6\text{H}_3-\text{CH}_2\text{CHCOOH}$ $\qquad \qquad $ $\qquad \qquad \text{NH}_2$	<i>3,4-Dihydroxyphenylpyruvic Acid</i> HO $\text{HO}-\text{C}_6\text{H}_3-\text{CH}_2\text{COCOCH}_3$	<i>Hydroxytyramine</i> HO $\text{HO}-\text{C}_6\text{H}_3-\text{CH}_2\text{CH}_2\text{NH}_2$
<i>Glutamic Acid</i> $\text{HOOCCH}_2\text{CH}_2\text{CHCOOH}$ $\qquad \qquad $ $\qquad \qquad \text{NH}_2$	<i>α-Ketoglutaric Acid</i> $\text{HOOCCH}_2\text{CH}_2\text{COCOCH}_3$	<i>γ-Aminobutyric Acid</i> $\text{HOOCCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$
<i>Glycine</i> CH_2COOH $ $ NH_2	<i>Glyoxylic Acid</i> OHCCOOH	<i>Methylamine</i> CH_3NH_2
<i>Histidine</i> $\text{CH}=\text{CCH}_2\text{CHCOOH}$ $ \qquad \qquad $ $\text{N} \qquad \qquad \text{NH}_2$ $\diagup \quad \diagdown$ CH	<i>β-Imidazolelactic Acid</i> $\text{CH}=\text{CCH}_2\text{CHOHCOOH}$ $ \qquad \qquad $ $\text{N} \qquad \qquad \text{NH}$ $\diagup \quad \diagdown$ CH	<i>Histamine</i> $\text{CH}=\text{CCH}_2\text{CH}_2\text{NH}_2$ $ \qquad \qquad $ $\text{N} \qquad \qquad \text{NH}$ $\diagup \quad \diagdown$ CH

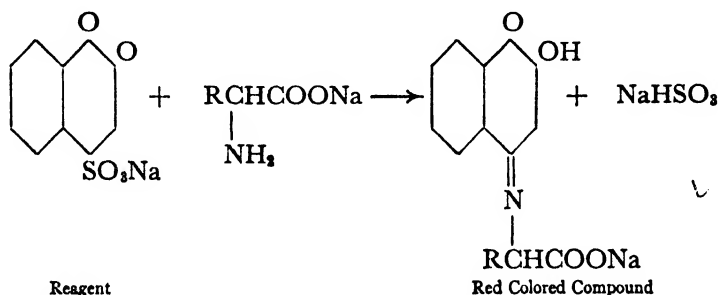
TABLE 67 (Cont.)

IMPORTANT BIOLOGICAL DEAMINATION AND DECARBOXYLATION PRODUCTS OF AMINO ACIDS

AMINO ACID	DEAMINATION PRODUCTS	DECARBOXYLATION PRODUCTS (APORRHOGMAS)
Leucine $\begin{array}{c} \text{CH}_3 \\ \\ \text{CHCH}_2\text{CHCOOH} \\ \quad \\ \text{CH}_3 \quad \text{NH}_2 \end{array}$	α-Ketoisocaproic Acid $\begin{array}{c} \text{CH}_3 \\ \\ \text{CHCH}_2\text{COCOOH} \\ \\ \text{CH}_3 \end{array}$	Isoamylamine $\begin{array}{c} \text{CH}_3 \\ \\ \text{CHCH}_2\text{CH}_2\text{NH}_2 \\ \\ \text{CH}_3 \end{array}$
Lysine $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHCOOH}$ $\quad \quad \quad $ $\quad \quad \quad \text{NH}_2$		Cadaverine $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$
Phenylalanine $\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{CH}_2\text{CHCOOH} \\ \\ \text{NH}_2 \end{array}$	β-Phenylpyruvic Acid $\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{CH}_2\text{COCOOH} \end{array}$	β-Phenylethylamine $\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{CH}_2\text{CH}_2\text{NH}_2 \end{array}$
Ornithine $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHCOOH}$ $\quad \quad \quad $ $\quad \quad \quad \text{NH}_2$		Putrescine $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$
Serine $\text{HOCH}_2\text{CHCOOH}$ $\quad \quad $ $\quad \quad \text{NH}_2$	Hydroxypyruvic Acid $\text{HOCH}_2\text{COCOOH}$	Ethanolamine $\text{HOCH}_2\text{CH}_2\text{NH}_2$
Tryptophane $\begin{array}{c} \text{C}_6\text{H}_4 \\ \\ \text{C}=\text{CH}_2\text{CHCOOH} \\ \quad \\ \text{CH} \quad \text{NH}_2 \\ \\ \text{NH} \end{array}$	β-Indolepyruvic Acid $\begin{array}{c} \text{C}_6\text{H}_4 \\ \\ \text{C}=\text{CH}_2\text{COCOOH} \\ \quad \\ \text{CH} \quad \quad \\ \\ \text{NH} \end{array}$	β-Indoleethylamine $\begin{array}{c} \text{C}_6\text{H}_4 \\ \\ \text{C}=\text{CH}_2\text{CH}_2\text{NH}_2 \\ \quad \\ \text{CH} \quad \quad \\ \\ \text{NH} \end{array}$
		Skatole $\begin{array}{c} \text{C}_6\text{H}_4 \\ \\ \text{C}=\text{CH}_2 \\ \quad \\ \text{CH} \quad \quad \\ \\ \text{NH} \end{array}$
		Indole $\begin{array}{c} \text{C}_6\text{H}_4 \\ \\ \text{CH} \\ \quad \\ \text{CH} \quad \quad \\ \\ \text{NH} \end{array}$
Tyrosine $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2\text{CHCOOH}$ $\quad \quad \quad $ $\quad \quad \quad \text{NH}_2$	p-Hydroxyphenylpyruvic Acid $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2\text{COCOOH}$	Tyramine $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2\text{CH}_2\text{NH}_2$
Valine $\begin{array}{c} \text{CH}_3 \\ \\ \text{CHCHCOOH} \\ \quad \\ \text{CH}_3 \quad \text{NH}_2 \end{array}$	α-Ketoisovaleric Acid $\begin{array}{c} \text{CH}_3 \\ \\ \text{CHCOCOOH} \\ \\ \text{CH}_3 \end{array}$	Isobutylamine $\begin{array}{c} \text{CH}_3 \\ \\ \text{CHCH}_2\text{NH}_2 \\ \\ \text{CH}_3 \end{array}$

is conducted at pH 5, carbon dioxide is freed quantitatively from all α -amino acids. The reaction provides a gasometric method for the determination of amino acids; aspartic acid gives two mols of carbon dioxide.

The formol, nitrous acid, and ninhydrin reactions are used for the determination of amino acids. A fourth procedure, Folin's colorimetric method, is based on the conjugation of the amino radical with 1,2-naphthoquinone-4-sulfonic acid in alkaline solution.

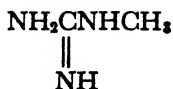


Similar red colors are given by certain alkaloids, amines, and ammonia; the latter can be separated from amino acids by means of permuted lime or by distillation. Amino acids can also be determined microbiologically by means of *L. arabinosus*, *L. casei*, or by other organisms for which amino acids are essential nutrients.

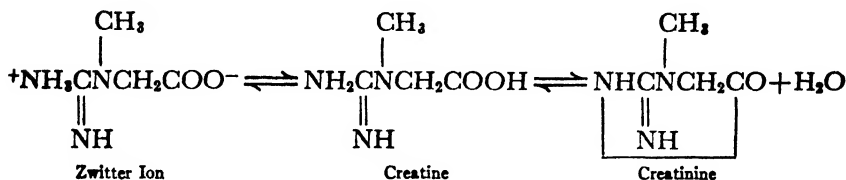
Methylation

Cells can readily methylate the nitrogen atoms of the amino acids and their decarboxylation products to produce methylamino acids, betaines, and methyl amines (Table 68). These substances constitute an important fraction of the non-protein *nitrogenous extractives* of tissues.

Sarcosine (methylglycine) and creatine are the most important *methyl-amino* acids in tissues; they are especially abundant in striated muscle. Betaine, glycine, taurine, and phosphoarginine are the chief extractives in invertebrate muscle; while in vertebrate muscle, phosphocreatine and carnosine (a peptide of histidine and β -alanine) predominate. Skeletal muscle also contains small quantities of carnitine, methylguanidine, and anserine (the methyl derivative of carnosine). Human striated muscle has from 350 to 400 mg. per cent *creatine*, present largely as phosphocreatine. Liebig's extract of beef contains more than 8 per cent free creatine. Creatine is a normal constituent of cells; but a derivative, methylguanidine,



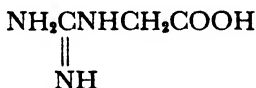
is toxic and produces convulsions when injected. Free creatine probably exists as a zwitter ion; heating, in acid solution, converts it almost quantitatively to the anhydride, *creatinine*.



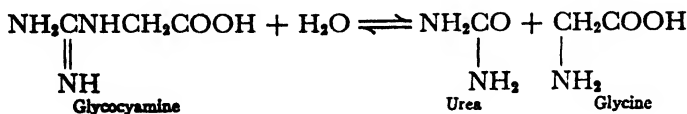
Alkali partially converts creatinine to creatine; when an alkaline solution of creatine is boiled, sarcosine, ammonia, and carbon dioxide are formed.

Creatinine is found in the body fluids and in urine. It is a more active reducing agent than is creatine, and it readily reduces alkaline copper reagents. Creatinine is stable in acid solutions. In alkaline picrate solution, it produces a characteristic red coloration, which is used for the quantitative determination of creatinine (Folin's method). Similar colors are given by diketopiperazines and by glycoyamidine. 3,5-Dinitrobenzoic acid can be used in place of picric acid for the colorimetric determination of creatinine. Creatine can be converted into creatinine, and the latter may then be determined by either of the above procedures. In alkaline solutions, creatinine reacts with sodium nitroprusside to give a red color which changes to yellow (Weyl's reaction). When the colored solution is acidified and heated, it becomes green owing to the formation of Prussian blue. The similar red color produced by acetone and nitroprusside changes to purple on being acidified.

The demethylation product of creatine, *glycoyamine* or guanidine acetic acid,

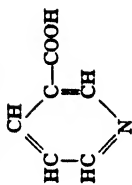


is regarded as the physiological precursor of creatine. Hydrolysis by acids or by the hepatic enzyme glycoyaminate converts glycoyaminate into urea and glycine:



The *betaines* are methyl derivatives of amino acids; they are classified as tetra-alkylammonium (R_4N^+) compounds. In alkaline solutions, they are converted to trimethylamine ($(\text{CH}_3)_3\text{N}$) and other substances. Ordi-

Nicotinic Acid



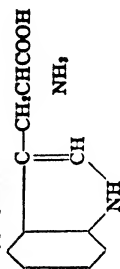
Phenylalanine



Proline



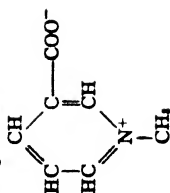
Tryptophan



Tyrosine



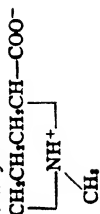
Trigonelline



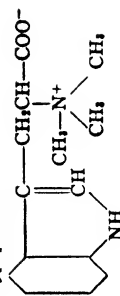
Ephedrine



Stachydrine



Hypaphorine



Adrenaline

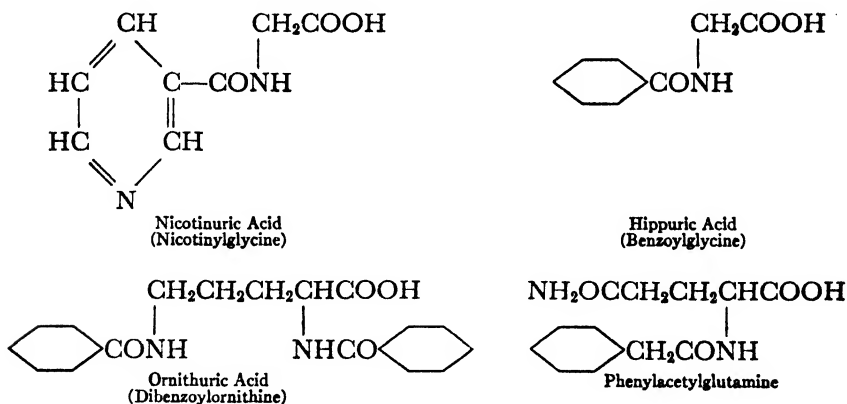


nary bêtaine, or trimethylglycine, $(\text{CH}_3)_3\text{N}^+ - \text{CH}_2\text{COO}^-$, is the acid corresponding to choline. It is a common constituent of plant tissues. Carnitine, myokinin, and trimethylglycine are found in muscles, ergothioneine in mammalian erythrocytes and in ergot, and trigonelline in plants and urine. The γ -butyrobetaine, or arrow poison, formed in putrefying meat by the decarboxylation and methylation of glutamic acid, has a paralytic curarine-like action on nerve endings. The betaines of the aromatic and the dicarboxylic amino acids are unstable.

Adrenaline and ephedrine are physiologically active *methylamines*; *l*-adrenaline is a hormone of the adrenal medulla, and a secretory product of the skin glands of poisonous toads. Physiologically, the *l*-isomer is fifteen times as active as its optical antipode. The chemistry of these methylamines is discussed on page 376.

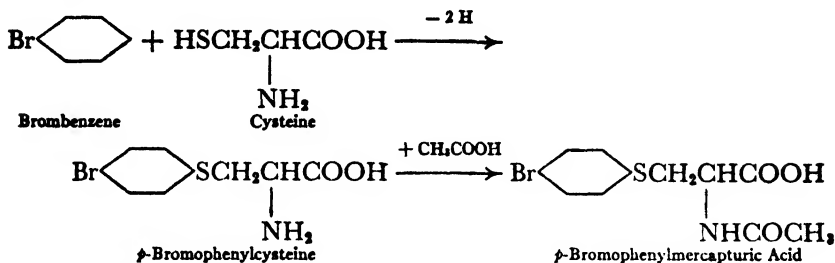
Acylation

In acylating reactions, the amino radicals of amino acids unite in peptide linkage with the carboxyl radicals of other acids. The conjugation of amino acids with aromatic and bile acids is discussed on pages 246 and 430. Excretory products of detoxifying acylations include ornithuric acid of birds, hippuric and nicotinuric acids of mammals, phenylacetylglutamine of primates, and phenylaceturic acid and mercapturic acid of dogs.

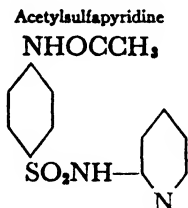


Hippuric acid is soluble in water, alcohol, ether, and ethyl acetate; it can be extracted from urine by either of the last two solvents. Heating dry hippuric acid decomposes it into glycine and benzoic acid; the mass turns red, and the benzoic acid sublimes. The hydrolysis of hippuric acid to glycine and benzoic acid can be effected by acids, by alkalies, and by the renal enzyme, histozyme. When a mixture of hippuric acid and concentrated nitric acid is evaporated, the characteristic odor of nitrobenzene can be detected.

In dogs, monohalogen derivatives of benzene are detoxicated by conjugation with cysteine, and subsequent acetylation of the products to form mercapturic acids.



Sulfonamide therapy of infectious diseases has led to increased interest in biological acetylation; the sulfonamide drugs are acetylated readily in the body. The sparingly soluble detoxication product, acetylsulfapyridine, can cause blockage of the urinary tract when excess sulfapyridine is administered, or when the fluid intake is inadequate.



The acetylation of toxins with ketene, $\text{CH}_2=\text{CO}$, reduces their toxicity (page 468).

Special Reactions of Individual Amino Acids

Glycine can be determined colorimetrically, after separation from tryptophane and ammonia, by the chloroform-soluble violet coloration which it gives with *o*-phthaldialdehyde. The determination of *alanine* is based on its oxidation by ninhydrin to acetaldehyde.

The *dicarboxylic acids* are found in proteins largely as the corresponding acid amides:

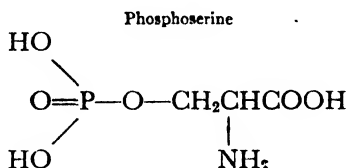


During the hydrolysis of proteins, the *amide nitrogen* of asparagine and glutamine is liberated as ammonia. Distillation of protein hydrolyzates with magnesium oxide *in vacuo* and determination of the ammonia in the distillate provides an approximate measure of the dicarboxylic acid content

of proteins. Free asparagine and glutamine are widely distributed in plant tissues. Aspartic acid can be determined by oxidation and deamination to a characteristic bromo derivative. Oxidation to succinic acid is the basis for glutamic acid determination.

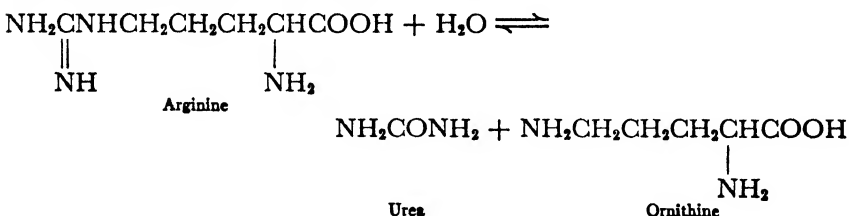
The *pyrrolidine amino acids* are actually imino acids. They can be determined colorimetrically by oxidation and condensation with isatin to form red colored complexes. Proline and hydroxyproline are convertible to glutamic acid through oxidation.

The *hydroxy amino acids* unite with phosphoric acid to form phosphate esters, such as phosphoserine which is a characteristic unit of the phosphoproteins.

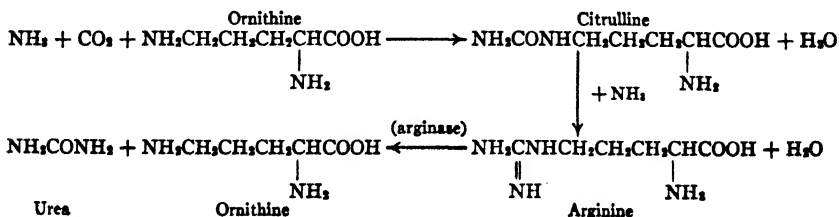


Alkali, or bone phosphatase, readily liberates phosphoric acid from phosphoserine and from peptones which contain this ester. Hydroxylysine, serine, and threonine can be determined by oxidation with periodate, which converts them to aldehydes, ammonia, and glyoxylic acid.

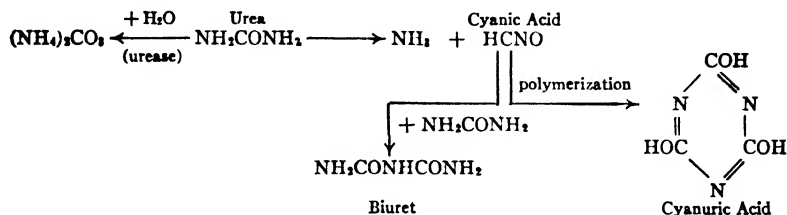
The basic amino acid, *arginine*, can be precipitated as the silver salt at pH 13.5. This amino acid is hydrolyzed to ornithine and urea by the enzyme, arginase, or by heating with alkali.



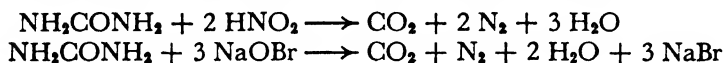
The most specific method for the determination of arginine is hydrolysis by arginase preparations, followed by estimation of the liberated urea. The ammonia produced by the biological deamination of amino acids appears as urea in the urine of mammals, amphibia, and most fishes. Urea is synthesized by the mammalian liver as follows:



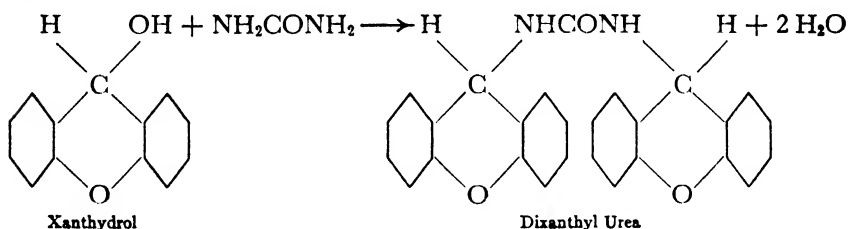
Urea, or carbamide, is a very soluble substance. Concentrated urea solutions have an extraordinary ability to disassociate proteins and polysaccharides (page 398). Urea is hydrolyzed to ammonium carbonate by acids and alkalis and by urease. When dry urea is heated, it forms biuret and cyanuric acid.



Urea reacts with nitrous acid and with alkaline hypobromite as follows:



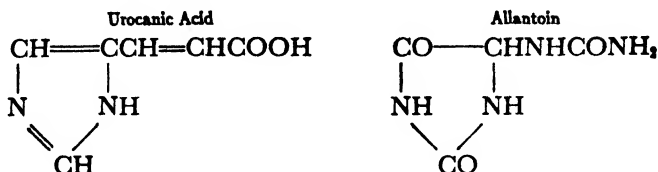
Measurement of the evolved nitrogen provides a rough method for the determination of urea. A more accurate procedure is hydrolysis by urease preparations and determination of the resulting ammonia by Nesslerization, or by distillation and titration. The enzyme, urease, is present in considerable quantities in Jack beans and soya beans, and in smaller amounts in bacterial cells. It is activated by sulphhydryl compounds, and inactivated by ascorbic acid or penicillin. Urea can be precipitated by xanthidrol.



This is a very sensitive reagent, but it also precipitates allantoin and allantoic acid. Nitric and oxalic acids form crystalline urea salts which are useful for microscopic identification.

Arginine and certain other *guanidine derivatives* react with α -naphthol and sodium hypobromite to produce a red coloration (Sakaguchi's test). This reaction can detect 0.04 mg. per cent of free arginine, but it is less sensitive to the combined arginine units in proteins. Methylguanidine, glycoamine, and angiotonin (page 459) give the Sakaguchi test. Guanidine derivatives give violet to pink colorations with biacetyl ($\text{CH}_3\text{COCOCH}_3$), in alkaline solution; this reaction has been used for the quantitative determination of arginine, citrulline, and creatine.

In animals and bacteria, *histidine* can be deaminated to urocanic acid; in rats, it also forms allantoin.



The amino acid has been regarded as a probable precursor of ergothioneine and of purines in certain animals. Histidine reacts with bromine water to give a red color, which turns purple on the addition of ammonia. After having been precipitated as the silver salt (at pH 7.4), histidine is determined colorimetrically by means of Koessler and Hanke's (or Pauly's) *p*-diazobenzenesulfonate reagent, which gives red colors with imidazole derivatives and with phenols. In the presence of oxygen and ascorbic acid, histidine is deaminated and its imidazole ring is ruptured.

The *aromatic amino acids* have several important color reactions. Tyrosine and tryptophane are responsible for the *xanthoproteic reaction* of proteins (the formation of a yellow precipitate or solution with concentrated nitric acid). The xanthoproteic precipitate tends to dissolve on being warmed, and it becomes orange colored when alkali is added. These colorations are due to the formation of nitro derivatives of tryptophane, tyrosine, and thyroxine. Diiodotyrosine, and purified gelatin and certain protamines which contain very small quantities of aromatic acids, give no xanthoproteic reaction. Phenylalanine gives the test only when the solid form is used.

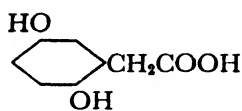
In animals, *phenylalanine* is readily oxidized to tyrosine. It can be determined colorimetrically by nitration and subsequent treatment with hydroxylamine in the presence of ammonia to produce the blue-violet ammonium salt of 3,4-dihydrodinitrobenzoic acid (Kapeller-Adler's method).

The phenolic amino acid, *tyrosine*, is responsible for the very sensitive *Millon's reaction* (the appearance of a red solution or coagulum on warming a protein with a nitric acid solution of mercury nitrites). The diluted reagent can be applied to insoluble proteins. Millon's test is not given by diiodotyrosine. The reagent is rendered insensitive by large concentrations of alcohol, alkali, peroxides, or inorganic salts. Since it reacts with phenols, it cannot be used to detect proteins in urine. Millon's reaction may be used for the quantitative determination of tyrosine (method of Folin and Ciocalteu). Tyrosine can also be determined colorimetrically by the phosphotungstic-phosphomolybdic acid reagent of Folin and Looney (after precipitating tryptophane with mercuric sulfate), or by Hanke and Koessler's diazotized sulfanilate reagent (after removing histidine as the silver salt). The Folin and Looney reagent reacts with both

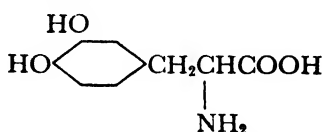
free and combined tyrosine, and it may be used for the determination of serum proteins. The tyrosine units are more reactive after denaturation of the protein.

The reagents mentioned above also react with *phenolic derivatives* of tyrosine, the most important of which are the following:

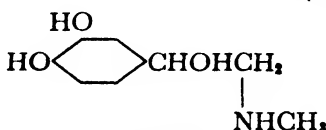
DIHYDRIC PHENOLS



Homogentisic Acid

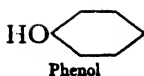


3,4-Dihydroxyphenylalanine
(Dopa)

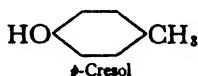


Adrenaline

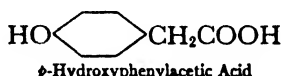
MONOHYDRIC PHENOLS



Phenol

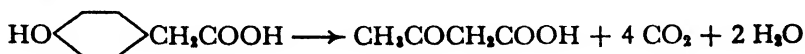


p-Cresol



p-Hydroxyphenylacetic Acid

Tyrosine and phenylalanine can be oxidized, biologically, to these phenols. Free phenols, which do not contain amino radicals, can be extracted with ether from aqueous solutions; they give characteristic colors with ferric chloride solution. The simpler monohydric phenols occur in urine, partly in the free form and partly as conjugated ethereal sulfates and glucuronides. *p*-Hydroxyphenylacetic acid is a postulated intermediate in the biological formation of acetoacetic acid from tyrosine.



Small quantities of *p*-hydroxyphenylacetic, *p*-hydroxyphenylpropionic, and *p*-hydroxyphenylglycolic acids are present in mammalian urine. All dihydric phenols are strong reducing agents and, in alkaline solution, they are auto-oxidizable.

The enzyme, tyrosinase, converts tyrosine into 3,4-dihydroxyphenylalanine, or dopa, which is an intermediate in the production of the insoluble brown or black *melanin pigments* of the skin, hair, iris, and choroid, and pigmented layer of the retina. The melanins are colloidal polymerized oxidation products of 3,4-dihydroxyphenylalanine, which is closely related to adrenaline and may be a precursor of this hormone. Melanins can also

be formed from adrenaline and tyramine by phenol oxidases. Typical melanin contains an indole nucleus formed by union of the α -amino nitrogen with the benzene ring (compare adrenochrome, page 106); other dark pigments classified as melanins do not contain nitrogen. In epidermal melanoblasts, melanin formation is accelerated by dopase; this enzyme is also present in the kidney and in the liver. Melanins are produced in considerable quantities by melanotic tumors. The presence of such tumors leads to the excretion of a urinary melanogen; the urine darkens on standing as the melanogen is oxidized to melanin. Melanogen reacts with the Obermayer and Jolle reagents (page 378), but it can be distinguished from indican by the insolubility of its oxidation product (melanin) in chloroform. The careful addition of ferric chloride solution to melanuric urine produces a dark precipitate; bromine water gives a yellow precipitate which gradually darkens.

Homogentisic acid is responsible for the darkening of the urine voided by patients with alcaptonuria or ochronosis. The darkening is due to oxidation of homogentisic acid as the voided urine becomes alkaline. This acid is supposedly formed from an isomer of dopa (2,5-dihydroxyphenylalanine). Homogentisic acid gives a blue color with ferric chloride, and it reduces alkaline copper reagents.

Adrenaline is oxidized rapidly by cytochrome and polyphenol oxidases, and a variety of laboratory oxidants to the red colored substance, adrenochrome, which does not possess adrenaline activity (page 106). Amine oxidase converts adrenaline to 3,4-dihydroxyphenylglycolic aldehyde. Light and alkali increase the sensitivity of adrenaline to oxidation. The catechol nucleus is responsible for the typical green coloration which adrenaline gives with ferric chloride in faintly acid solution (Vulpian reaction). Adrenaline can be determined colorimetrically by reduction of Folin's uric acid reagent. This method is not specific, and biological assay by means of blood pressure or uterine contraction measurements is more satisfactory.

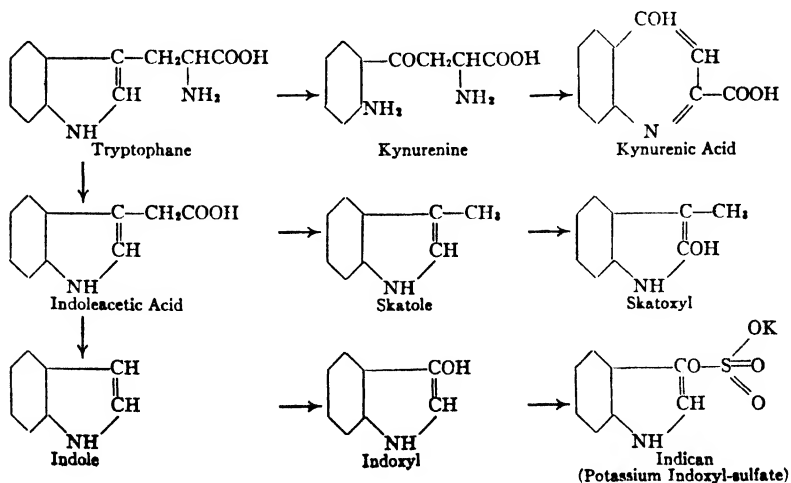
Iodogorgoic acid, or 3,5-diiodotyrosine, is found in the natural skeletal substances of sponges and corals and in artificially iodized proteins. Both diiodotyrosine and its derivative, thyroxine, are present in thyroglobulin (the hormone of the thyroid). Diiodotyrosine and thyroxine have been isolated from thyroglobulin in quantities representing one third and one sixth of the iodine content, respectively. Thyroxine and diiodotyrosine are the physiologically active units of thyroglobulin. They can be formed in casein by treating this protein with iodine. Such iodized proteins have physiological activity similar to that of thyroglobulin. Thyroxine can be produced from free diiodotyrosine by warming it in slightly alkaline solution. Free diiodotyrosine is without thyroxine activity. Acid hydrolysis removes iodine from both these iodo amino acids. 3,5-Dibromotyrosine is found in the horny skeletal proteins of certain corals.

Tryptophane is destroyed rapidly by acids and by sodium hydroxide, but

it can be recovered quantitatively from protein hydrolyzates prepared with barium hydroxide or with enzymes. The hydrolysis of proteins by acids other than hydriodic acid transforms tryptophane to brown-black *humins*; this reaction is caused by the condensation of aldehydes with tryptophane at the α -position of its indole nucleus. The principal aldehydes concerned in humin production are the furfurals liberated from prosthetic carbohydrate radicals of the proteins. By providing an excess of tryptophane, the formation of humin becomes a quantitative measure of prosthetic carbohydrates (page 295). The humin nitrogen produced by the hydrolysis of proteins varies from 0 to 10 per cent of the total nitrogen.

A specific test for indole derivatives, including tryptophane, is the *glyoxylic acid* or *Hopkins-Cole* reaction. A solution of glyoxylic acid (OHCCOOH) is mixed with a protein or tryptophane solution and stratified over concentrated sulfuric acid; a violet ring appears. The reaction is not given by gelatin and certain protamines, which do not contain tryptophane units. The color development is prevented by nitrate, nitrite, and excess chloride. *Ehrlich's reagent* (*p*-dimethylaminobenzaldehyde) is a fairly specific reagent for indole derivatives, but it also reacts with urobilinogen. In the presence of strong hydrochloric acid, *p*-dimethylaminobenzaldehyde gives blue colors with tryptophane and skatole, and red colors with indole and indoleacetic acid. Tryptophane can be determined quantitatively by Ehrlich's reagent, and also by the Folin reagents for tyrosine (after separation of the tryptophane with mercuric sulfate). Free tryptophane gives a violet color with bromine in slightly alkaline solution; this test is useful for estimating the extent of protein digestion.

In certain mammals, the tryptophane units of protein are oxidized to a quinoline derivative, kynurenine acid. The acid and its intermediate



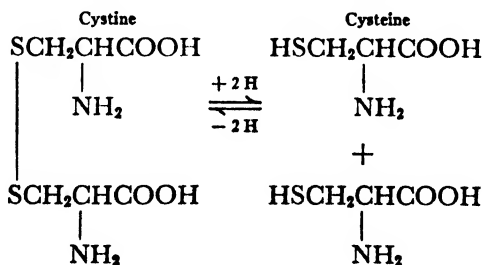
metabolite, kynurenine, are excreted in the urine. Kynurenine is the v^+ gene hormone (page 496), and the precursor of ommochrome pigments (ommatus and ommins) which are widely distributed in insects. During pyridoxin deficiency, mammals convert kynurenine to xanthurenic acid (4,8-dihydroxy-2-quinoline carboxylic acid), and excrete it in the urine. This acid gives a green color with ferric chloride.

Indoleacetic, indolepropionic, indolebutyric, phenylacetic, anthraceneacetic, α -naphthaleneacetic, and fluoreneacetic acids are *heteroauxins* or growth hormones of plants (page 713). Indoleacetic acid is a bacterial metabolite; it is also found in plants, yeasts, molds, and mammalian urine. It reacts with nitrous acid to produce a reddish substance, uro-rosein.

The tryptophane aporrhemas, *indole* and *skatole*, are oxidized by animals to the corresponding phenols (indoxyl and skatoxyl). The latter are partly conjugated with sulfuric and glycuronic acids prior to excretion in the urine. Indole has a fecal odor; when an alkaline solution of indole is treated with 1,2-naphthoquinone-4-sulfonic acid and the solution is shaken with chloroform, a red color is imparted to the chloroform (Herter's test). Indican can be roughly estimated in urine by oxidation to indigo blue, and extraction of this pigment with chloroform. The oxidizing agents commonly employed are Obermayer's reagent (0.3 per cent ferric chloride solution in concentrated hydrochloric acid), and Jaffe's reagent (calcium hypochlorite and concentrated hydrochloric acid). Even more sensitive is Jolle's reaction, which employs 0.5 per cent ferric chloride in fuming hydrochloric acid and an alcoholic thymol solution. The chloroform extract has a violet color. Indican can be determined quantitatively either by these reactions or by the red coloration which it gives with ninhydrin.

Sulfur-Containing Amino Acids

Cystine and cysteine constitute an important reversible oxidation-reduction system (page 104). They are mutually interconvertible, whether in the free state or as units of peptides and proteins.



Cystine can be reduced to cysteine by such mild reducing agents as hydriodic acid, thioglycolic acid, cyanides and sulfides, or by excess of

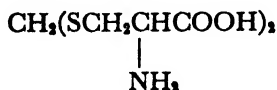
sulfhydryl compounds. In feebly alkaline solution cysteine is oxidized to cystine by ferricyanide, porphyrindin, tetrathionate, and also by oxygen in the presence of copper or iron salts. These metals form auto-oxidizable coordination compounds with cysteine; cyanides inhibit their oxidation. Iodine will oxidize cysteine in acid solution. The reactivity of sulfhydryl radicals is inhibited similarly by iodoacetates. Sulfhydryl amino acids are decomposed by alkali, with the formation of hydrogen sulfide. The presence of sulfur-containing amino acid units in proteins is shown by the formation of the dark-colored lead sulfide when an alkaline protein solution is heated with a little lead acetate. Cystine is precipitated, together with the basic amino acids, by phosphotungstic acid in hydrochloric acid solution. In a strongly reducing medium, Folin's amino acid reagent (page 366) reacts specifically with cysteine. This reagent is used in Sullivan's method for the quantitative determination of cysteine; cystine can be determined similarly, after reducing it to cysteine. In Okuda's method, cystine is reduced to cysteine and the latter is titrated with potassium iodate in the presence of potassium iodide. Reduced sulfhydryl compounds (cysteine, reduced glutathione, and certain proteins) give a characteristic temporary reddish purple coloration with sodium nitroprusside in ammoniacal solution; the reaction is due to the formation of $\text{Na}_4[\text{Fe}(\text{CN})_6\text{NSO}]$. Some of the sulfhydryl radicals of proteins are relatively unreactive with nitroprusside, ferricyanide, and porphyrindin reagents, but can react with iodoacetamide and iodine. A portion of the cystine in proteins is convertible by alkali to lanthionine (cystine minus one sulfur atom).

Cysteine can be oxidized by bromine to a sulfonic acid (cystic acid). In animals, cystic acid is decarboxylated to taurine, which is the chief nitrogenous extractive of the muscles of certain marine invertebrates and an important constituent of conjugated bile acids.

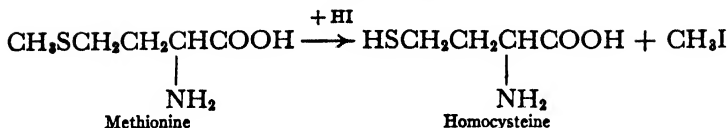


In animals, the sulfur of amino acids is normally oxidized to sulfate which is excreted in the urine.

The alkylated sulfur found in methionine and in djenkolic acid,



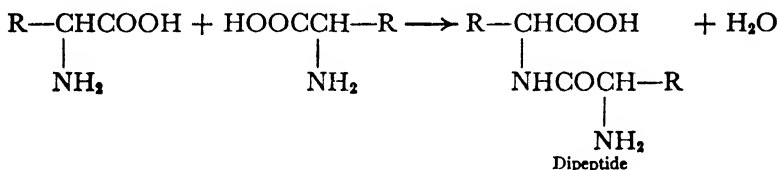
is much less reactive than the sulfhydryl radical. The methyl radical of methionine can be removed by hydrolyzing with hydriodic acid.



This reaction is utilized for the determination of methionine in proteins; after the protein is hydrolyzed with hydriodic acid, the homocysteine is determined by means of tetrathionate.

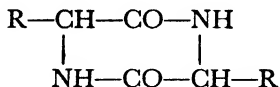
PEPTIDES

Amino acids unite in peptide linkage ($-\text{NH}-\text{CO}-$) by the elimination of water between the amino radical of one amino acid and the carboxyl radical of another.



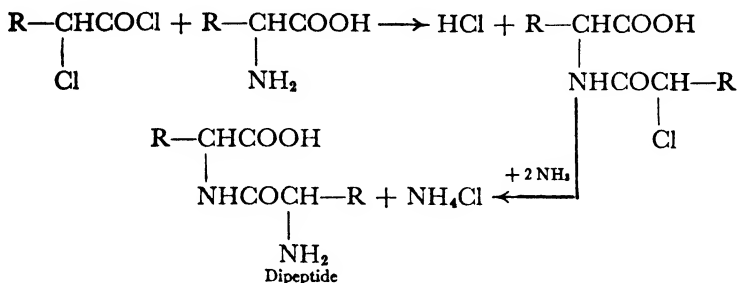
Compounds which contain such substituted amide linkages are called peptides; they are classified as dipeptides, tripeptides, and polypeptides according to the number of amino acid units. Peptides with three or more amino acid units give the *biuret reaction*, a very sensitive general test for proteins. In this reaction, a violet to purple coloration is produced by the addition of very dilute cupric sulfate solution to an alkaline protein solution. Pink colors are characteristic for peptides, peptones, and proteoses. In the presence of large concentrations of magnesium or ammonium sulfate, considerable alkali is required for the biuret reaction; strongly alkaline protamines give the test without added alkali. The biuret reaction is due to the formation of complex copper-peptide coordination compounds. Similar colorations are given by certain non-protein substances which contain two amino radicals, also by free asparagine, biuret, histidine, serine, and threonine.

Diketopiperazines

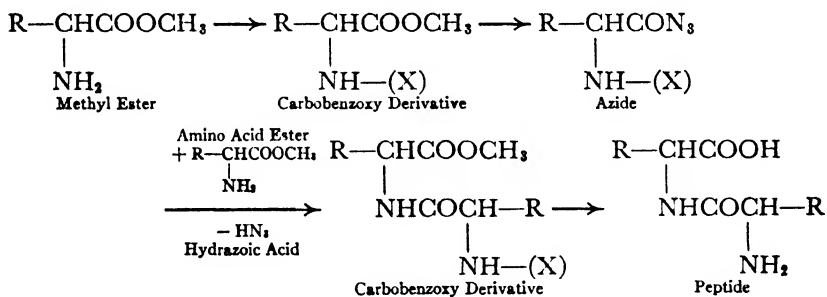


are cyclic amino acid compounds which contain double peptide linkages. They are not demonstrable in native proteins or in polypeptides, but are formed by the dehydration of peptides during hydrolysis of proteins. A few peptides can be prepared by the partial hydrolysis of diketopiperazines, or by the condensation of amino acid esters. Many have been isolated

from partially hydrolyzed protein, and some have been synthesized by a reverse action of proteolytic enzymes in the presence of cysteine. The historic Fischer halogenacyl synthesis of peptides may be represented by the following reactions:



This method is limited to the synthesis of peptides of monoamino acids; it causes partial racemization. The modern carbobenzoxy synthesis is more generally applicable, and avoids racemization. In the carbobenzoxy method, the amino acids are transformed to esters; the amino radicals are masked by carbobenzoxy radicals, and the carbobenzoxy derivatives are converted to azides by successive treatment with hydrazine and nitrous acid. These azides are then combined with the amino radicals of other amino acid esters to form peptide linkages; the carbobenzoxy radicals are later removed by catalytic hydrogenation.



(X) is the carbobenzoxy radical, $-\text{OCOCH}_2$

STRUCTURE OF PROTEINS

The fundamental feature of protein structure is a folded polypeptide chain of considerable length; from 55 to 75 per cent of the nitrogen of proteins is peptide nitrogen. Individual amino acids tend to be repeated periodically in natural polypeptide chains, so that an amino acid recurs

TABLE 69

A. SUGGESTED FREQUENCIES OF AMINO ACID UNITS
IN PROTEINS¹

AMINO ACID	INSULIN ²	OVAL- BUMIN	FIBRIN (Cattle)	HEMO- GLOBIN (Cattle)	KERATIN (Wool)	FIBROIN (Silk)
Alanine					18	4
Arginine	48	28	18	48	16	216
Aspartic acid		15	18	18	16	
Cysteine	8	60	64	192	9	
Glutamic acid	4	8	8	36	9	
Glycine					9	2
Histidine	12	96	48	18	192	2592
Leucines	4			4	9	64
Lysine	96	26	12	16	48	648
Methionine		26	48		192	
Phenylalanine					36	128
Proline			18	48	16	
Serine					9	8
Threonine	12				16	
Tryptophane		140	32	144	96	
Tyrosine	12	40		48	36	16
Valine					24	
Total amino acid units ³	288	386	576	576	576	2592

AMINO ACID	EDESTIN	GELATIN	β -LACTO- GLOBULIN	MYOSIN (Rabbit)	SECRETIN	YELLOW ENZYME
Alanine		9		15		9
Arginine	9	18	54	21	18	18
Aspartic acid	9		12	13	36	36
Cysteine	72		96	72		288
Glutamic acid	6		6	6	36	16
Glycine		3		34		
Histidine	54		90	82	36	48
Hydroxyproline		9				
Leucines		18				
Lysine	54	24	13	12	12	9
Methionine	54		41	38	36	
Phenylalanine						24
Proline		6			18	
Serine				25		48
Threonine				27		
Tryptophane	108		94	192		36
Tyrosine	36		42	44		18
Total amino acid units ³	432	216	324?	576?	36	576

TABLE 69 (Cont.)

B. RATIOS OF BASIC AMINO ACIDS ¹

	ARGININE	HISTIDINE	LYSINE
Hemoglobins ⁵	3 (12)	8 (32)	9 (36)
Insulin	2 (6)	8 (24)	1 (3)
Hemocyanins	1	1	2
Secretin	(2)	(1)	(3)
Fibrin	8 (32)	3 (12)	12 (48)
Neurokeratin	3	1	3
Ovalbumin	(14)	(4)	(15)
Skin keratin	7	1	6
Eukeratin (hair, nails)	12 (36)	1 (3)	4 (12)
Fibroin	(12)	(1)	(4)
Edestin	6 (48)	1 (8)	1 (8)
Gelatin	4 (12)		3 (9)
β -Lactoglobulin	(6)	(3.6)	(25)
Myosin	(27.4)	(7)	(48)
Serum γ -globulin (human)	(46)	(28)	(78)
Serum albumin (human)	(25)	(16)	(28)
Yellow enzyme	8 (32)	3 (12)	16 (64)
Cytochrome <i>c</i> ⁶		(3)	(22)
Clupein ⁷	(20)		
Salmin ⁸	(24)		
Gramicidin ⁹			

¹ Interpretation: The figure 18 for aspartic acid, for example, indicates that this amino acid constitutes each eighteenth unit along the polypeptide chain.

² Zinc = 96.

³ The total number of amino acids divided by the frequency value gives the molecular ratio of the amino acids.

⁴ The molecular ratios or amino acid units per protein molecule are given in parentheses.

⁵ Of cattle. For horse, human, and sheep hemoglobins, the arginine values are (14), (16), and (15), and the histidine values are (33), (35), and (32), respectively.

⁶ Cytochrome *c* contains 96 amino acid units.

⁷ Clupein contains 30 amino acid units, including 4 valine and 2 each of alanine, proline, and serine.

⁸ Salmin contains 36 amino acid units, including 6 proline, 4 serine, and 2 valine.

⁹ Gramicidin contains 24 amino acid units, including 6 leucine, 6 tryptophane, 5 valine, 2 glycine, and 2 hydroxyamino acid.

at positions having a characteristic frequency (Table 69). However, x-ray analysis and determination of the quantities of individual amino acid units indicate that some proteins (keratin, β -lactoglobulin, myosin, ovalbumin) do not display strict periodicity. Such proteins may contain several polypeptide chains of differing composition, or there may be irregular condensations of certain amino acid units along the polypeptide

chain. Suggested total numbers of amino acid units are: fibroin, 2592; human serum γ -globulin, 1525; human serum albumin, 618; fibrin, globin, keratin, and yellow enzyme, 576 each; edestin, 432; ovalbumin, 386; β -lactoglobulin, 324; insulin, 288; gelatin, 216. The average molecular weights which correspond to polypeptide chains of 288, 576, and 864 amino acid units are 35,000, 70,000, and 105,000, respectively (Table 71, page 397).

X-ray and electron microscope studies, and other evidence, indicate two general types of arrangement of the polypeptide chains in the protein megamolecules or micelles: a fibrous or rodlike type, in which folded or spiral chains lie roughly parallel to the fiber or megamolecule axis; and a globular type, in which the polypeptide chains are in non-parallel configurations that give the molecule an ellipsoidal shape. Only Bence-Jones protein and erythrocrucorin molecules have been found to be perfectly spherical. Examples of fibrous proteins include collagens, erythrocyte stroma protein, fibrin, fibroin, keratins, myosin, nucleoproteins (including a number of viruses), and scleroproteins. Many soluble native proteins, such as Bence-Jones protein, bushy stunt virus, erythrocrucorin, gliadins, insulin, hemocyanins, hemoglobins, β -lactoglobulin, myoglobin, ovalbumin, pepsin, serum albumins, serum γ -globulin, and trypsin, are globular or ellipsoidal in type but they can assume fibrous patterns when denatured (page 399). The number of free α -amino radicals present in globular proteins, and evidence from x-ray diffraction studies, suggest a laminated structure of superimposed polypeptide subunits for these molecules. Estimates of the number of laminar subunits per molecule are: β -lactoglobulin, 8; ovalbumin, 4; insulin, 18; hemoglobin, 16; human serum γ -globulin, 25 (with 60 amino acid units each); and the serum albumins of cattle, horse, and man, 39 (with 16 amino acid units each), 35 (with 18 amino acid units each), and 36 (with 17 amino acid units each) respectively.

The type and position of the *grid linkages* connecting the polypeptide chains in the protein micelles are important determinants of protein configuration. The chains can be bound through residual or secondary valencies (coordination linkages and hydrogen bridges), polar salt unions between basic and dicarboxylic amino acid units, and —S—S linkages of cystine units.¹ The ϵ -amino radical of lysine, and the β - and γ -carboxyl

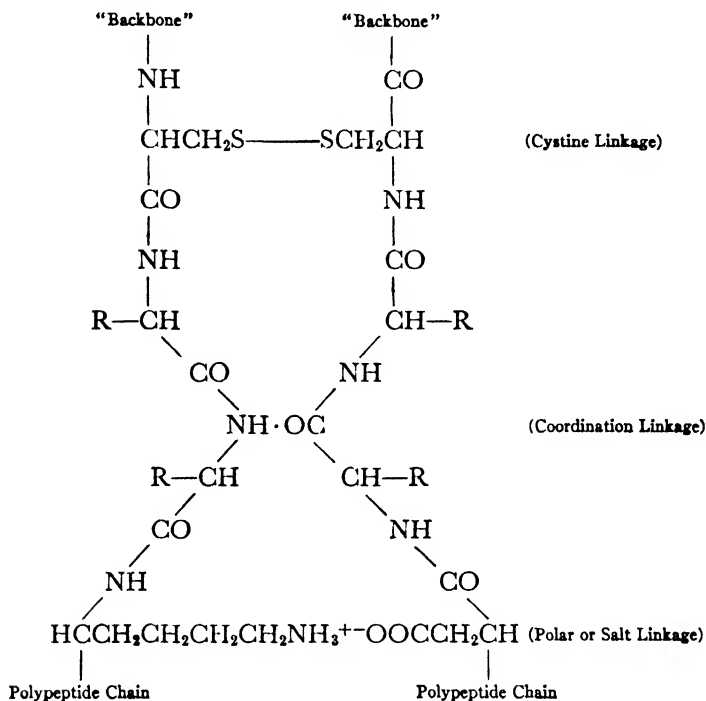
¹ Chemical bonds may be classified as:

Ionizable or polar bonds, which involve electron transfer, represented by + and - signs.

Covalent, non-ionizable or electron-sharing bonds, indicated by a solid line.

Coordination bonds (usually covalent in character), represented by a broken line, dotted line, or arrow. These bonds include residual valences and van der Waal's forces. The two chief types of coordination linkages are the *hydrogen bond* (first harmonic of the vibration of a hydrogen atom linked to fluorine, nitrogen, or oxygen atom), and the *Werner bond* (increase in valence of a central atom). The latter alters the oxidation-reduction potential of the central metal ion, and leads to stabilization of an unusual valence. Formation of a ring by coordination linkage is termed *chelation*. Coordination with water molecules to form aquo complexes is known as *aquation*.

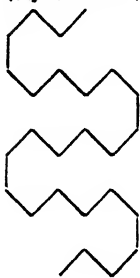
radicals of aspartic and glutamic acids do not participate in the main peptide chains, but are active in forming grid linkages. The grid linkages produce definite space arrangements, as can be seen from the diagram:



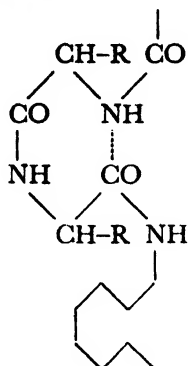
The stabilities of the several grid linkages differ; keratin molecules are very stable, whereas the serum proteins are readily disassociated into the fundamental polypeptide chains and the latter are easily reoriented into new arrangements (page 398). In fibrous proteins, the coordination or accessory valence grid linkages between neighboring —NH—CO— radicals are rather stable; grid linkages are also formed between $>\text{NH}$ and —OH radicals of proteins.

The folded polypeptide chains of such fibrous proteins as keratin, fibrinogen, and myosin are hexagonal molecular springs which can be stretched to longer denatured modifications. The potential elasticity of the above named proteins is apparently related to alternation of the side chains in direction and type (polar or non-polar). In changing from the α - to the β -form, keratin elongates about 100 per cent. The extended β -form can return to the more folded α -configuration when allowed to relax in the presence of polar molecules.

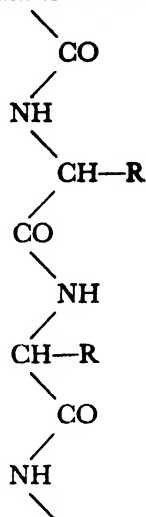
MYOSIN
(Supercontracted)



α -KERATIN, α -MYOSIN



β -KERATIN, β -MYOSIN
(Stretched or Denatured)



The chains of fibrin, denatured seed globulins, and silk fibroin exist in the extended β -form; the collagen group of proteins shows about 80 per cent of the maximal extension. X-ray diagrams of muscle fibers indicate that a folded structure, similar to that of α -keratin, is normal for myosin; this protein can undergo further folding, as in muscular contraction, to a supercontracted state in which the parallel grid arrangement is disturbed. Such reversible intramolecular transformations are of considerable biological significance. When proteins are spread as molecular films on water, the peptide linkages and the free amino and carboxyl radicals are in the water surface, while other portions of the polypeptide chains project outward. In this condition, the protein configuration is distorted and partial denaturation occurs (page 399).

Asymmetric and anisotropic molecular patterns are characteristic for fibrous proteins, certain ellipsoidal proteins, and lipides. Marked differences in the molecular axes cause *optical anisotropy* or double refraction of light. Smooth muscle and the dark Q bands of striated muscle are highly anisotropic; as the muscle begins to contract, there is a sudden decrease in double refraction. Anisotropy of muscle fibers is related to the parallel arrangement of the long fibrous myosin micelles. In flowing solution, this protein exhibits orientation of its asymmetric particles and double refraction (which disappears on denaturation). Stretched artificial fibers of myosin are elastic, undergo heat contracture, and present x-ray diagrams similar to those of muscle. Other proteins which exhibit appreciable optical anisotropy (often only in flowing solutions) include collagen, fibrino-

gen, gelatin, hemocyanin, livetin, ovoglobulin, thyroglobulin, and virus nucleoproteins. All these proteins have elongated micelles.

Oxidation causes closure of —S—S— linkages between cysteine units of proteins; the linkages can be ruptured by reducing systems or by alkali. Certain native proteins, such as thymus histone, give direct nitroprusside reactions for —SH radicals; crystallin, edestin, excelsin, globin, and ovalbumin do so only after denaturation; and serum albumin after reduction by cyanide, hydrogen sulfide, or thioglycollic acid. Scission of —S—S— grid linkages produces structural alterations in proteins; insulin and a number of the anterior lobe pituitary hormones are physiologically active only in the —S—S— form, while the —SH modifications of certain proteolytic enzymes constitute the active catalysts (page 84). Keratins, which contain large proportions of cysteine units, are reduced by thioglycollic acid and sulfides, with rupture of —S—S— grid linkages. The resulting fragments, termed kerateines, are soluble in acids and alkalies, and are digested by proteolytic enzymes. Reoxidation of the kerateines does not reunite the fragments in the specific manner required for keratin formation; instead, soluble and digestible stereoisomers are produced.

INDIVIDUAL PROTEINS

The characteristics of seven groups of simple proteins and of five types of conjugated proteins are summarized in Table 70.

The *simple proteins* are classified largely on the basis of solubility and flocculation. Albumins and globulins are important *heat-coagulable proteins*, which constitute the bulk of the cytoplasmic proteins in animal and plant cells. While these are the principal heat coagulable proteins, hemoglobin, certain metaproteins, and the porphine chromoproteins can also be coagulated by heat. The *albumins* are found in largest proportions in the fluids of animals, and the *globulins* preponderate in the cells. Serum globulin can be separated from serum albumin by flocculating the former with one-half saturated ammonium or sodium sulfate. The ordinary serum albumin and serum globulin fractions are mixtures. By electrophoresis in slightly alkaline solution, serum globulin has been separated into α -, β -, and γ -fractions which have similar molecular weights but varying isoelectric points, as indicated in Table 10, page 49. (See also page 396.) As the salt concentration is increased gradually, there is a stepwise flocculation of the plasma proteins in the following order: fibrinogen, γ -globulin, β -globulin, α -globulin, albumins. Fibrinogen and euglobulin may be fractionated from pseudoglobulin by flocculation with one-third saturated ammonium or sodium sulfate. The pseudoglobulin fraction is soluble in water, and in saturated sodium chloride solution. Certain pseudoglobulins are flocculated only at the salt concentrations characteristic for albumins. Euglobulin and similar animal globulins can be flocc-

culated by carbon dioxide, dialysis, or dilution with water. Euglobulin is probably a conjugated lipoprotein compound of pseudoglobulin and phospholipide. The reserve globulins of seeds and nuts occasionally exist in native crystalline form. These plant globulins tend to give atypical reactions; some are soluble only in hot salt solutions, and they are not as readily salted out as the animal globulins; others are imperfectly coagulated by heat. Flocculation by one-half saturated ammonium sulfate is not a specific characteristic of globulins inasmuch as gelatins, globins, hemoglobins, metaproteins, mucoids, phosphoproteins, porphine chromoproteins, virus nucleoproteins, primary proteoses, Bence-Jones protein, and certain plant albumins are flocculated under the same conditions.

The *prolamines* and *glutelins* are important dietary proteins of cereal seeds. The *scleroproteins* are extracellular fibrous proteins formed by animal cells. The *histones* and *protamines* are markedly basic intracellular proteins.

The *conjugated proteins* are compounds of simple proteins with such prosthetic substances as lipides, nucleic acids, nucleotides, phosphoric acid, pigments, or polysaccharides. The *nucleoproteins* are protein salts of nucleic acids (page 513). They are prominent constituents of the chromatin of cell nuclei. α -Nucleoproteins of animal tissues are extracted by water, at room temperature, and are flocculated by acetic acid; the β -nucleoproteins are extracted by boiling water. The prosthetic radicals of α - and β -nucleoproteins are desoxyribonucleic acid and ribonucleic acid, respectively. The relations of nucleic acid prosthetic radicals to staining have been discussed on page 78. Flavoproteins are chemically related to nucleoproteins, since the flavin-phosphate prosthetic radicals resemble nucleotides. These colored proteins constitute important cellular oxidation-reduction systems (page 102).

The *glycoproteins* are compounds of proteins with the complex prosthetic polysaccharides considered on page 294. The term glycoprotein should be restricted to proteins which contain considerable polysaccharide. Numerous albumin, globulin, collagen, casein, and vitellin preparations have less than 5 per cent polysaccharide, which, in some instances, is due to contamination with mucoids. Thus, globoglycoid and seroglycoid are largely responsible for the polysaccharide found in ordinary serum globulin and serum albumin fractions. Mucoid is present in largest quantity in the serum albumin fraction; it is not coagulated by heat. The ovomucoid of egg white flocculates on dilution with water. The animal sulfomucins (formerly termed mucins) are acidic protein compounds whose solubilities resemble those of nucleoproteins and phosphoproteins. The prosthetic polysaccharides of the sulfomucins are closely related to the heparins. The polyuronide acids of mucins and sulfomucins are united in salt linkage to basic amino acid units of the proteins.

The *phosphoproteins* are typical constituents of milk and egg yolk; only

small quantities are detectable in cells. They are acidic proteins; hence, in milk, casein exists largely as calcium salts.

With the exception of the hemocyanins and chlorocruorins, the *chromoproteins* are intracellular substances. Their prosthetic radicals include such pigments as porphines, porphyrins, metal-peptide complexes, and carotenoids. The chromoproteins play important catalytic roles in aerobic oxidation (pages 97 and 98). The porphyrin prosthetic radicals are united by coordinate linkage to nitrogenous radicals of globins. Hemocuprin, the prosthetic radical of hemocyanins, is a copper-peptide complex which contains arginine, leucine, tyrosine, and considerable sulfur. Rhodopsin, or visual purple, has about ten prosthetic carotenoid radicals per molecule.

The *lipoproteins* exist in all cells, but few have been characterized exactly. The prosthetic lipides are not extracted by ether until the non-polar coordination linkages with protein are ruptured by alcohol, bile salt, or urea solutions.

The chemistry of the nucleic acids, and the porphine and porphyrin prosthetic substances, will be discussed in Chapter VII.

GENERAL PROPERTIES OF PROTEINS

Proteins share the general optical and amphoteric properties, the deamination, methylation, and acylation reactions described for amino acids. The simple proteins are levorotatory, while nucleoproteins and hemoglobins are dextrorotatory. Proteins respond to the formol titration, Van Slyke's nitrous acid deamination, and the ninhydrin and biuret reactions; they also give the special analytical tests for the individual amino acids which they may contain. Although the amide and peptide nitrogen of proteins can combine with formaldehyde in acid solution, only the amino radicals are involved in the formol titration. Proteins are typical hydrophilic colloids and are, therefore, flocculated by concentrated electrolytes. (See footnote to Table 70 for salting out of proteins.) Proteins can be removed from solution by adsorption on alumina cream or on colloidal iron. Many proteins form gels.

TABLE 70

✓ CLASSIFICATION OF PROTEINS,¹ II

II. <i>Simple proteins</i>	Contain chiefly amino acid units, or their derivatives.
<i>Albumins</i>	Soluble in water and dilute electrolytes; coagulated by heat. Examples: antithrombin (blood plasma), crystalalbumin (serum albumin), lactalbumin (milk), leucosin (cereals), insulin ² (pancreas), ovalbumin (egg white), myogen (vertebrate muscle sarcoplasma), ricin (castor bean), ribonuclease.
<i>Globulins</i>	Insoluble in water; soluble in dilute electrolytes; flocculated by dialysis and by half-saturation with ammonium sulfate; globulins of animal origin are coagulated by heat.

TABLE 70 (Cont.)

CLASSIFICATION OF PROTEINS, II

<p><i>Examples:</i> alkaline phosphatase (kidney) amandin (almond), arachin (peanut), concanavalin (Jack bean), α- and β-crystallin (lens), edestin (hemp, and cereal seeds), excelsin (Brazil nut), fibrinogen (blood plasma), globulin X (muscle), glycinin (soya bean), growth hormone (pituitary), hepatoglobulin (liver), hypertensinogen (blood plasma), legumin (pea), lactoglobulin (milk), livetin (egg yolk), myosin (muscle sarcostyles), neuroglobulin (nerve), pepsin (gastric juice), phaseolin (bean), polysaccharide phosphorylase (muscle), prolactin (pituitary), serum globulins, thyroglobulin (thyroid), thyrotropin (pituitary), trypsin (pancreatic juice), tuberin (potato), urease (Jack bean).</p>	
Prolamines (gliadins) . . .	Insoluble in water; soluble in dilute acid and alkali, and in 70 to 80 per cent alcohol. <i>Examples:</i> avenin (oat), gliadin (wheat), hordein (barley), papain (papaya), secalin (rye), zein (corn).
Glutelins	Insoluble in water; soluble in very dilute acid or alkali. <i>Examples:</i> glutenin (wheat), oryzenin (rice).
Scleroproteins (albuminoids) .	Insoluble fibrous proteins that form supportive structures in animals. <i>Examples:</i> chondroalbuminoid (cartilage), collagens, conchiolin (mussel and snail), elastins, fibroin (silk), gorgonin (coral), haversian albuminoid (bone), ichthylepidin (fish scale), keratins, keratohyalin, neurokeratin (medullary sheath), ossealbuminoid (bone), reticulin (reticular tissue), spongin (sponge).
Collagens	Proteins of vertebrate bone, cartilage, connective tissue, and skin; also present in cephalopods; digested by pepsin; resist digestion by trypsin; converted into tough non-putrefying leather by tannic acid, etc.; changed to digestible gelatins upon boiling with water or dilute acid; form typical gels.
Gelatins	Soluble in dilute salt solution; form heat-reversible gels; flocculated by half-saturation with ammonium sulfate.
Elastins	Proteins of arteries, tendons, and yellow connective tissues; digested by pepsin or trypsin.
Keratins	Proteins of epidermal structures of birds and mammals; resist digestion by pepsin and trypsin.
Eukeratins	Keratins of hair, feathers, wool, nails, horn, etc.; histidine, lysine, and arginine present in ratios of 1 : 4 : 12.
Pseudokeratins	Keratins of human skin, turtle scutes, neurokeratins, etc.; low arginine content.
Histones	Soluble in water and dilute acid; flocculated by ammonia, alkalis, and proteins; flocculated by nitric acid with resolution upon warming; basic, owing to large proportions of basic amino acid units; exist largely in combination with nucleic acids and heme derivatives. <i>Examples:</i> globins, nucleohistones.
Globins	Histones which exist in combination with heme; flocculated by half-saturation with ammonium sulfate; coagulated by heat in the presence of half-saturated ammonium sulfate; only denatured globins are insoluble in alkali.
Protamines ^a	Soluble in water and dilute acid; flocculated by other proteins; strongly basic, owing to large proportions of basic amino acid units; exist largely in combination with nucleic acids. <i>Examples:</i> clupein (herring), cyprinin (carp), percin (perch), salmin (salmon), scombrin (mackerel), sturin (sturgeon), thynnin (tuna).

TABLE 70 (Cont.)

CLASSIFICATION OF PROTEINS, II

III. <i>Conjugated proteins</i>	Contain both amino acid units and prosthetic radicals.
<i>Nucleoproteins</i>	Compounds of histones, protamines, or other proteins with nucleic acids or nucleotides; insoluble in water and dilute acetic acid; soluble in dilute alkali; contain 4 to 6 per cent phosphorus; ⁴ nucleic acids removed by acid or alkali. <i>Examples:</i> flavoproteins (yellow enzymes), dehydrogenases (pyridine nucleotide proteins), thymus nucleoprotein, viruses, yeast nucleoprotein.
<i>Flavoproteins</i>	Protein salts of flavinphosphoric acids. <i>Examples:</i> Amino acid oxidases, cytochrome reductase, diaphorase, glucose oxidase, glycine oxidase, xanthine oxidase.
<i>Glycoproteins</i>	Compounds of proteins with polysaccharides; soluble in water and dilute alkali; less soluble in dilute acids; polysaccharides removed by boiling with alkali.
<i>Mucoids</i>	Compounds of proteins with neutral polysaccharides; some resemble globulins in their solubilities. <i>Examples:</i> bacterial proteins, cornea mucoid, salivary mucoid, gastric mucoid, gonadotropins, avidin (egg white), globoglycoid (blood plasma), ovomucoid (egg white), prothrombin (blood plasma), seroglycoid (blood plasma), thrombin (blood plasma), vitellomucoid (egg yolk).
<i>Mucins</i>	Compounds of proteins with hyaluronic acid. <i>Examples:</i> bacterial proteins; certain glycoproteins accompanying the sulfomucins in the umbilical cord, mucous secretions, ocular and synovial fluids, and connective tissue.
<i>Sulfomucins</i> ⁶	Compounds of proteins with sulfuric esters of polyuronides; flocculated by dilute acetic acid.
<i>Chondroproteins</i>	Glycoproteins of mesodermal tissues, bone, cartilage, sclera, tendon; present in the matrix between collagen fibers; prosthetic substance is chondroitin sulfuric acid. <i>Examples:</i> chondromucoid, osseomucoid, tendomucoid, amyloid (degenerated tissue).
<i>Mucoproteins</i>	Glycoproteins of mucosae, cornea, ovarian cysts, and amphibian integument; flocculates are slimy; prosthetic substance is mucoitin sulfuric acid. <i>Examples:</i> gastric mucin, submaxillary mucin, heparin proteins.
<i>Phosphoproteins</i>	Phosphoric esters of proteins, the phosphoric radical being combined with hydroxy amino acid units (phosphoserine); insoluble in water or dilute acids; soluble in dilute alkali; flocculated by half-saturated ammonium sulfate; contain about 0.9 per cent of phosphorus; phosphoric acid removed by warming with alkali. <i>Examples:</i> casein (milk), vitellin (egg yolk), ichthulin (fish eggs).
<i>Chromoproteins</i>	Compounds of proteins (histones or globulins) with pigments; prosthetic substances removed by heating with acid or alkali.
<i>Porphyrin compounds</i>	Contain iron-porphyrin complexes.
<i>Hemoglobins</i>	Red pigments of vertebrate erythrocytes; heme compounds of globins; soluble in water; coagulated by heat; flocculated either by saturation or half-saturation with ammonium sulfate, according to species; heme removed by addition of acid or alkali.
<i>Hemochromogens</i>	Yellow to red heme compounds of proteins, other than globin. <i>Examples:</i> catalase and peroxidase of numerous cells; erythrocruorins of invertebrate corpuscles; myoglobins of striated muscle.
<i>Other porphyrin pigments</i>	<i>Examples:</i> green chlorocruorins of invertebrate plasma, cytochrome <i>c</i> , cytochrome oxidase.

TABLE 70 (Cont.)

CLASSIFICATION OF PROTEINS, II

<i>Porphine compounds</i> . . .	Contain bile pigment radicals, but no iron or copper; insoluble in water; soluble in electrolytes; flocculated by acetic acid, or by half-saturation with ammonium sulfate; coagulated by heat. <i>Examples:</i> blue phycocyan and red phycoerythrin of algae.
<i>Non-porphine chromoproteins</i>	
<i>Iron compounds</i>	Contain hemoferrin prosthetic radicals. <i>Examples:</i> hemerythrins (red pigments of the corpuscles of worms).
<i>Hemocuprin compounds</i> .	Contain copper-peptide prosthetic complexes (hemocuprin). <i>Examples:</i> hemocyanins (blue pigments of the sera of crustacea and molluscs).
<i>Cupreins</i>	Have copper-containing prosthetic radicals of unknown constitution. <i>Examples:</i> hemocuprein (erythrocytes), hepatocuprein, polyphenol oxidases, tyrosinase.
<i>Carotenoid compounds</i> . . .	<i>Examples:</i> ovoverdin, contains astacin ester prosthetic radical (lobster eggs); iodopsin or visual violet, prosthetic radical is a vitamin A derivative (cones of the retina); porphyropsin and rhodopsin, contain retinene as the prosthetic radical (visual purples of rods of retina).
<i>Lipoproteins</i>	Compounds of proteins with phospholipides and sterides; solubility and flocculation vary with the nature of the protein constituent; lipides removed by strong alcohol, bile salt, or urea solutions. <i>Examples:</i> euglobulin (serum), β -globulin (serum), lecithovitellin (egg yolk), lipoalbumin (serum), thromboplastins.

¹ All proteins, proteans, metaproteins, and proteoses are flocculated by saturation with ammonium sulfate, or by saturation with sodium chloride after the addition of acetic acid. With few exceptions, saturation with sodium sulfate is equivalent to saturation with ammonium sulfate; while saturation with sodium chloride or magnesium sulfate is equivalent to half-saturation with ammonium sulfate. For saturating 100 ml. of protein solution the following quantities of salts are required: ammonium sulfate, 80 gm.; magnesium sulfate .7 H₂O, 102 gm., sodium chloride, 36 gm. anhydrous sodium sulfate, 44 gm.

² Insulin is unusually soluble in alcoholic solutions.

³ While protamines are usually grouped with proteins, they have low molecular weights and are closely related to proteoses in many respects

⁴ Some virus nucleoproteins have only 0.4 to 0.6 per cent phosphorus.

⁵ Formerly termed mucins.

Amphoteric Properties

The basic properties of proteins are due largely to free ϵ -amino, guanidine, and imidazole radicals of lysine, arginine, and histidine, respectively. Proteins give the Sakaguchi reaction for uncombined guanidine nuclei; the acid-binding capacity of clupein is exactly equivalent to its arginine content. The free ϵ -amino radicals of lysine units, and the free α -amino radicals of the terminal amino acids of the polypeptide chains, react with nitrous acid. Van Slyke's method indicates the following numbers of free amino radicals per molecule of protein: serum γ -globulin (human), 103; serum albumin (human), 64; carbonylhemoglobin, 53; β -lactoglobulin, 36; cytochrome *c*, 32; ovalbumin, 24; insulin, 21; pepsinogen, 17; zein, 12; edestin, 9; pepsin, 5; and elastin, 2. The large proportions of basic amino acid units present in the protamines endow these proteins with markedly basic properties and the ability to unite readily with other proteins to form

salts such as protamine-insulin. The basic radicals make it possible for a protein to combine with acids at a pH zone below the isoelectric point, where the protein exists as a cation. Only acids which have ionization constants greater than those of the protein carboxyl radicals can form salts readily with proteins. Hence, most polybasic acids combine with proteins in equimolecular proportions. Sulfuric acid is an exception, owing to its low pK_2 (Table 1, page 8). Proteins are flocculated by chromic, ferricyanic, metaphosphoric, nitric, phosphomolybdic, phosphotungstic, picric, sulfosalicylic, tannic, trichloroacetic, and tungstic acids, and by certain dyes. Tungstic and trichloroacetic acids are used widely to deproteinize biological fluids and tissue extracts for quantitative analysis. Nitric acid, sulfosalicylic acid and sodium chloride-acetic acid solution are employed as flocculating reagents in the detection of urinary albumin (page 445).

Proteins contain large proportions of dicarboxylic amino acid units, but they are only weakly acidic. This is due to the fact that their non-peptide carboxyl radicals are largely in the form of acid amides (glutamine and asparagine). The small residue of free carboxyl radicals allows proteins to form salts with cations, alkaloids, and basic dyes at a pH zone above the isoelectric point, where the protein exists as an anion. The alkaline earths and heavy metals form protein complexes which are not ionized. Heavy metal cations form insoluble compounds with proteins; these can redissolve in the presence of an excess of the metallic salt, since soluble complexes are then formed. Multivalent cations and anions, such as Al^{+++} , Ce^{++++} , La^{++++} , Th^{++++} , and $citrate^{---}$, can combine with proteins over a wide pH range, which extends across the isoelectric point.

The isoelectric points of most proteins are between pH 4 and pH 7 (Table 10, page 47). However, the isoelectric points for certain glycoproteins, nucleoproteins, pepsin, and silk fibroin are in the range, pH 2.75 to pH 3.5; while those for avidin, collagen, cytochrome, histones, papain, peroxidases, protamines, ribonuclease, secretin, and trypsin are high (pH 7.5 to 12.2). Isoelectric proteins exist largely as dipolar ions. Inorganic impurities can be removed by washing insoluble isoelectric proteins with distilled water, or by dialyzing or electro-dialyzing solutions of isoelectric proteins. Further purification is effected by crystallization at the isoelectric point, where proteins tend to be least soluble; ammonium sulfate is often added further to decrease solubility and to encourage crystallization. Albumins, hemoglobins, and trypsin have been crystallized by these methods; urease crystallizes from 30 per cent alcohol solution, and plant globulins crystallize during dialysis.

The dipolar ions of isoelectric proteins carry as many negative as positive charges, but the pH of a pure protein solution is seldom 7. The proteins exist largely as anions and carry excess negative charges at pH 7. At their isoelectric points the proteins show minimal combination with cations or anions, conductivity, membrane potential, osmotic pressure, swelling, and viscosity. The Donnan membrane potentials of protein systems are

determined largely by the hydrogen ion concentration; at either side of the isoelectric point, these forces increase from zero to maxima and then decrease again as the ionization of the protein changes with the pH. There are also optimal pH zones for swelling; the maxima for gelatin are at pH 3 and 10.5, while keratin swells below pH 1 and above pH 11. In compact tissues and such rigid protein systems as wool and hair, the pH has relatively little influence on swelling.

In solution, proteins usually contain about 10 to 20 per cent of water of hydration. The influences of electrolytes on swelling are related to the Donnan equilibrium. Protein gels and crystals swell because of the osmotic forces of the protein ions and crystalloids trapped within the gels. Neutral salts diminish the osmotic pressure and swelling of a protein, except at the isoelectric point; but they depress membrane potentials only at a given pH. The valence of a diffusible electrolyte associated with the protein is important in such equilibria. Polyvalent cations can break the dipolar ion balance and render a protein membrane positive at the alkaline side of the isoelectric point by forming complex ions. The relations of the Hofmeister or lyotropic series of ions to swelling and flocculation are outlined in Table 11, page 50. These effects are conspicuous in concentrated salt solutions, but they tend to disappear on dilution. The Hofmeister series roughly parallels the hydration and valency of ions. Salting out is usually maximal at the isoelectric point, and its efficiency increases with the molecular weight and non-polar radical content of the protein. The solvent action of salts on globulins increases with valency and, vice versa, serum albumin and hemoglobin increase the solubility of neutral salts. Such phenomena and the salting out of proteins are, in the last analysis, chemical effects.

DETERMINATION OF PROTEINS

Micro modifications of the Kjeldahl method are used for the quantitative determination of proteins in biological fluids. The protein solution is heated with concentrated sulfuric acid, in the presence of a catalyst or 30 per cent hydrogen peroxide. The protein nitrogen is liberated quantitatively as ammonia, and the latter may be determined either by distillation and titration or colorimetrically by Nesslerization (development of an orange coloration by adding alkaline $\text{HgI}_2 \cdot 2 \text{KI}$ solution). The nitrogen found is multiplied by 6.25 (100/16) to calculate the quantity of protein. This calculation is based on the fact that average animal and plant proteins contain 16 ± 2 per cent of nitrogen. It is not valid for ferritin (8.4 per cent nitrogen), mucoids and sulfomucins (10.5 to 13.5 per cent nitrogen), edestin and fibroin (19 per cent nitrogen), or protamines (21 to 32 per cent nitrogen).

The modified Kjeldahl method is applicable to the determination of biological non-protein nitrogenous substances. To determine the total

non-protein nitrogen (NPN), the biological fluid is first deproteinized and the filtrate is analyzed by the Kjeldahl method. Since compounds which contain alkylated or cyclic nitrogen are converted to a mixture of alkylamines and ammonia, they cannot be determined quantitatively by Nesslerization. Biological substances of this type include betaines, choline, creatine, creatinine, the decarboxylation products of several amino acids and certain pyridine, pyrrol, and quinoline derivatives.

MOLECULAR WEIGHTS

Minimal molecular weight values for certain proteins can be calculated from the content of inorganic elements, or of certain amino acid units, provided these are present in small proportions (e.g., copper, iron, phosphorus, sulfur, zinc, cystine, hexone bases, tryptophane, tyrosine). The copper content is only 0.17 to 0.38 per cent in hemocyanins, 0.34 per cent in cupreins, and 0.2 to 0.34 per cent in polyphenol oxidases and ascorbic acid oxidase. Ferritin, hemoglobins, and cytochrome *c* have 20, 0.34 and 0.43 per cent of iron, respectively, while catalase and peroxidases have 0.1 per cent. Carbonic anhydrase contains 0.33 per cent of zinc, and zinc-insulin has 0.5 per cent. The sulfur content of most proteins is from 0 to 3 per cent, although some keratins have as much as 8 per cent. The molecular weight values calculated from such factors are usually fractions of the true molecular weights. Values calculated from physical measurements are more reliable.

The osmotic pressure of a protein system at equilibrium may be used to calculate the molecular weight, provided corrections be applied for the ionization of proteins. Osmotic pressure measurements show that the molecular weight of the hemoglobins from cattle, horses, men, and sheep is four times that calculated from the iron content. Hence, hemoglobin has four atoms of iron in its molecule. Osmotic pressure measurements of high molecular proteins, such as hemocyanins and viruses, are inaccurate, and they are affected greatly by the presence of low molecular impurities.

The ultracentrifuge is a valuable instrument for the determination of the molecular weights of protein micelles. Centrifugal force up to four hundred thousand times gravity is employed to sediment the protein particles from solution. Since diffusion counteracts the centrifugal sedimentation, the rate of sedimentation is an indirect measure of the rate of diffusion, and, hence, of the molecular weight. Determinations of sedimentation equilibria are made at high speeds in order to shorten the time required for the determination, and thus to minimize the decomposition and denaturation of the proteins. The boundary between the sedimenting protein and the solvent is recorded photographically, ultraviolet light being used for colorless proteins. Homomolecular proteins are those which give sharp boundaries, while polydisperse proteins show more diffuse boundaries. When mixtures of soluble protein molecules are separated by the

ultracentrifuge, they present a stepwise appearance in the sedimentation photographs. High speed centrifugation is an effective modern method for separating and purifying protein preparations.

Table 71 contains a summary of the molecular weights of proteins, calculated from sedimentation velocity, diffusion, and osmotic pressure. Values for the smallest typical protein molecules are in the region of 17,600 (0.5 old Svedberg unit or 1 revised Svedberg unit, equivalent to approximately 144 amino acid units) (page 384). The ultracentrifuge gives somewhat unsatisfactory molecular weight values for proteins which have less than 4 Svedberg units or 576 amino acid units. Osmotic pressure measurements give lower and, perhaps, more accurate values for these smaller molecules. Values for the molecular weights of individual proteins having the same number of Svedberg units vary with the amino acid composition of the proteins. The ovalbumin molecule has a diameter of 43.5 Å (Ångström units), or 4.35 μ ; the diameter of the edestin molecule is about 78.8 Å or 7.88 μ ; and hemocyanins have diameters of 80 to 170 Å or 8 to 17 μ . In general, the globular protein molecules have diameters greater than 35 Å, while single polypeptide chains are about 10 Å in thickness. A more complex unit, whose molecular weight is approximately 400,000, has been suggested for the high molecular proteins.

Studies of molecular weights have shown that customary casein, gelatin, lactalbumin, and myoglobin preparations, and certain keratins, nucleoproteins, prolamines, and protamines are polydisperse mixtures (Table 71). Some proteins, like ovalbumin, serum albumin, and serum globulin, which appear to be homomolecular, can be separated into several components by electrophoretic methods. The electrophoretic fractions are either single proteins or mixtures of similarly charged proteins. The speed of electrophoretic migration varies with the pH, the activity of the electrolyte present, and at times with the shape and size of the protein particles. Electrophoresis separates plasma proteins into the following fractions (in order of decreasing mobility in the electric field): albumins (including follicle-stimulating hormone), α -globulins (including α_1 and α_2 fractions, alkaline phosphatase, complement endpiece, hypertensinogen, lipoproteins, pseudo-globulins, and thyrotropin), β -globulins (including β_1 and β_2 fractions, complement midpiece, fibrinolysin, isohemagglutinins, lipoproteins, luteinizing hormone, prothrombin, and thrombin), fibrinogen, and γ -globulins (including antibodies, euglobulins, and luteinizing hormone). In its native state serum globulin is apparently homomolecular, but it can be changed readily to euglobulins and pseudoglobulins through laboratory manipulations. This native protein mixture may be regarded as a protein-protein complex, joined through secondary valences. Demonstration of protein mixtures by the ultracentrifuge and electrophoresis nullifies the older chemical estimates of the molecular weights of proteins.

TABLE 71

APPROXIMATE MOLECULAR WEIGHTS OF PROTEINS

Pitocin	1,300	Diphtheria antitoxin	90,500
Pitressin	1,300	Lactoperoxidase	92,700
Gramicidin	1,400	Concanavalin A	96,000
Tyrocidine	2,500	Chorionic gonadotropin (human)	100,000
Cobra neurotoxin	3,000	Luteinizing hormone (pig)	100,000
Clupeins	4,450	Nucleoprotein antigen (hemolytic strepto-	
Artefolin	5,000	coccus)	100,000
Pepsin inhibitor	5,000	Zymohexase	100,000
Secretin	5,000	Canavalin	113,000
Trifidin	5,000	Gelatins	10,000-150,000
Salmin	5,600	Myogen	150,000
Trypsin inhibitor	6,000	Globulin X	160,000
Tuberculin (bovine)	10,000	Serum globulin ⁹	165,000
Cytochrome <i>c</i> ¹	13,000	Peroxidases	35,000-175,000
Ribonuclease	13,000	Serum γ -globulin	176,000
Tuberculin (human)	14,500	Pseudoglobulin (serum)	142,000-177,000
Histones	17,000	Diphtheria antibody	184,000
Lysozyme	17,700	Pneumococcus antibody ⁸	165,000-195,000
Corticotropin (sheep)	20,000	Fibroin (silk)	217,000
Lactogenic hormone (sheep)	22,000	Catalase ¹⁰ (beef liver)	225,000
Lactalbumins	12,000-25,000	Catalase ¹⁰ (horse)	248,000
γ -Chymotrypsinogen	27,000	Phycocyan	270,000
Glialin	27,000	Visual purple	270,000
Papain	27,000	Yellow enzymes ¹¹	38,000-280,000
Scarlet fever toxin	27,000	Phycocerythrin	290,000
Hordein	27,500	Excelsin	295,000
Carbonic anhydrase	30,000	Ovoverdin	300,000
β -Chymotrypsinogen	30,000	Edestin	310,000
Crotoxin	30,000	Amandin	330,000
Pituitrin	31,000	Caseins	75,000-375,000
Trypsin	34,000	Foot and mouth virus	400,000
α -Bence-Jones protein	35,000	Phosphorylase (muscle)	340,000-400,000
Hemocprein ²	35,000	Urease	483,000
Lactogenic hormone (cattle)	35,000	Apoferitin	500,000
Insulin ³	35,500	Fibrinogen	500,000
β -Bence-Jones protein	37,700	Influenza A virus	650,000
Pepsin	38,000	Thyroglobulin	650,000
α -Chymotrypsinogen	40,000	Poliomyelitis virus	700,000
Luteinizing hormone (sheep)	40,000	Pneumococcal antibody ¹²	930,000
Secalin	40,000	Nucleohistone (thymus)	2,100,000
Trypsinogen	40,000	Erythrocytorins ¹¹	17,100-3,150,000
β -Lactoglobulin	41,600	Chlorocruorin	3,400,000
Concanavalin B	42,000	Myosin	3,900,000
Pepsinogen	42,000	Yellow fever virus	4,300,000
Peroxidase II	44,100	Hemocyanins ¹¹	397,000-9,660,000
Ovalbumin ⁴	44,500	Bushy stunt virus	10,600,000
Zein ⁴	45,000	Genes (calculated maximum)	33,000,000
Ovomucoid	49,300	Tobacco mosaic virus	43,000,000
Fetuin	51,000	Rabbit papilloma virus	47,000,000
Globin (horse)	66,500	Rous sarcoma virus	142,000,000
Myoglobins	16,900-68,000	Equine encephalomyelitis virus	152,000,000
Keratin (wool)	68,000	Thromboplastin (cattle lung)	167,000,000
Hemoglobin ⁴	69,000	Bacteriophages ¹¹	400,000-500,000,000
Fibrin	69,300	Influenza virus	700,000,000
Serum albumin ⁷	70,000	Rabies virus	800,000,000
Diphtheria toxin	74,000	Herpes simplex virus	1,400,000,000
Carboxylase protein	75,000	Vaccinia virus	2,300,000,000
Cytochrome <i>c</i> reductase	75,000	Psittacosis virus	8,500,000,000

¹ Contains 1 atom of iron.

² Contains 2 atoms of copper.

³ Contains 3 atoms of zinc.

⁴ Molecular weight of 65 per cent of the zein mixture.

⁵ Older values and osmotic pressure measurements give 35,000.

⁶ Same for dog, horse, man, ox, sheep, also for carbonyl- and oxy-hemoglobins; contains 4 atoms of iron.

⁷ Same for horse, human, ox, and sheep.

⁸ Types I and III sera from human, monkey, and rabbit.

⁹ Same for human and horse.

¹⁰ Contains 4 atoms of iron.

¹¹ Values vary with species or type.

¹² Types I and III sera from cow, horse, and pig.

ASSOCIATION AND DISASSOCIATION

The combination or polymerization of polypeptide chains, through residual valencies, is termed association; hence, the fragmentation of the high molecular protein polymers may be termed disassociation (as distinguished from dissociation or ionization). These processes are reversible, particularly in the protein systems of milk and serum, and in the gliadins, hemocyanins, myoglobins, and virus nucleoproteins. Acids, alkalies, and concentrated salt solutions readily cause rearrangements within the systems; and the fractions isolated by means of such reagents may present variable compositions. It has been suggested that the associated coagulable serum protein system (*i.e.*, the native complex of serum albumin and serum globulin fractions) be termed orosin.

The molecular weights of polymerized proteins are frequently simple multiples of their disassociated forms. Dilution with water causes some disassociation in protein solutions, while concentration or salting out by ammonium sulfate tends toward association. It is, therefore, possible that more albumin and less globulin are present in native serum than in the isolated fractions. The presence of a second protein or polypeptide can induce disassociation, an effect which may explain the existence of a serum orosin of relatively low molecular weight in undiluted serum. Many proteins are stable, or homomolecular, between pH 4 and pH 9, but become polydisperse beyond these limits, or in the presence of electrolytes. In alkaline solution, proteins are readily disassociated into fragments approximating lower multiples of the Svedberg unit. High concentrations of urea, or other amides, disassociate such proteins as amandin, edestin, excelsin, hemocyanins, hemoglobin, myogen, myosin, and virus nucleoproteins; the molecules of hemoglobin and myogen are divided into halves, of edestin into fourths, and of amandin and excelsin into sixths. This action of urea is closely allied to denaturation.

DENATURATION

Various tissue proteins are coagulated by heat in aqueous solution, but not in the dry state. Heat coagulation consists of two reactions, namely, denaturation, and flocculation or coagulation of the denatured protein. Isoelectric denatured protein tends to be insoluble in water and in salt solutions. Denaturation represents a chemical alteration of protein structure induced by acids, alkalies, alcohol, acetone, bile salts, detergents, guanidine salts, heavy metals, sodium salicylate, potassium iodide, thiocyanates, urea, heat, x-rays, ultraviolet light, visible light in the presence of photosensitizers, high pressure, adsorption at a surface, or mechanical agitation. In the absence of such factors, denaturation is a very slow reaction. Denaturation of trypsin by heat, of pepsin by alkali or heat, and of hemoglobin by sodium salicylate (as well as the denaturation of chymo-

trypsin, pepsinogen, and thyroglobulin), is completely reversible. An equilibrium exists between the native and the denatured forms of any one of these proteins. Ordinary heat flocculation of denatured albumins and globulins is irreversible. There is approximately a six hundred fold increase in the rate of denaturation for each 10° C. increase in temperature. Hence, denaturation is very rapid above 60° C. in neutral solutions. Each protein has a fairly characteristic coagulation temperature, which may be lowered by acids or alkalis. While isoelectric coagulated proteins are usually insoluble in water, they dissolve in sufficiently concentrated solutions of salicylates or urea. The insoluble metaproteins (Table 64, page 351), produced by the action of acid and alkali, have properties which resemble those of heat-denatured proteins.

Denaturation and coagulation of proteins frequently increase the number of —SH radicals detectable by the nitroprusside reaction. This is true for ovalbumin. Liberation of masked —S—S—, and —SH linkages by opening or extension of the protein configuration occurs during denaturation by alkali, cyanides, sulfides, and thioglycolates. Changes in molecular weight are not essential to denaturation, and the —S—S— grid linkages are not changed to —SH linkages (as they are in the disassociation of keratins to kerateines; page 387). Moderate concentrations of urea disassociate hemoglobin, but do not denature it. The fact that the rate of denaturation is independent of protein concentration is further evidence that the number of molecules remains unchanged, and that the linkages which are ruptured during denaturation are internal or ring linkages.

The structure of denatured protein is less specific than that of native protein; during denaturation, the polypeptide chains of globular proteins unfold with a consequent loss of the original specific configurations. Crystalline protein enzymes become inactive on denaturation, immunological specificities of proteins disappear, and insulin and anterior lobe pituitary hormones are inactivated by denaturing reducing agents. Native hemoglobin is not digested by trypsin, but becomes digestible when it is denatured; similarly, the digestibility of ovalbumin is increased by denaturation. Denatured proteins are difficult to crystallize, even though crystalline proteins can be denatured without loss of form. Denatured globins are less firmly combined with heme, and these hemochromogens no longer combine reversibly with oxygen. Other prosthetic radicals are also dissociated from conjugated proteins by heat denaturation.

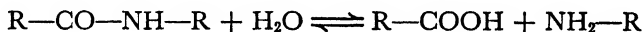
Proteins tend to spread as insoluble elastic monomolecular films at interfaces, including the surfaces of bubbles produced by shaking protein solutions. The maximal spreading occurs at the isoelectric point of the protein. The flat polypeptide films are only 10 Å thick; hence, the polypeptide chains of globular proteins uncoil, and fibrous characteristics become intensified. This intramolecular change leads to denaturation; and, therefore, films of pepsin and urease are partially inactivated. Heat

denaturation is maximal in acid and alkaline solutions, whereas surface denaturation is maximal at the isoelectric point. Proteins which are soluble in water have hydrophilic polar radicals at the surface, and hydrophobic radicals in the interior of the molecule. When the proteins unfold to produce films, the polar hydrophilic radicals remain in the water, and the hydrophobic portions become oriented in the non-aqueous phase.

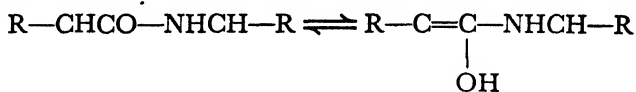
Denaturation apparently occurs in certain physiological processes. The photochemical reactions in the retina involve liberation of the carotenoid prosthetic radical of visual purple, following the denaturing action of light. Rigor of muscles represents a change of myosin to an insoluble form. Myosin is denatured by heat in two stages; the first corresponds to thermal contraction of muscle, and the second to heat rigor or complete denaturation. In muscular contraction there is a folding of myosin polypeptide chains rather than true denaturation (page 386). Normal muscle plasma clots slowly on standing, but this change is attributed to slight hydrolysis of myosin and myogen to the corresponding insoluble proteans.

HYDROLYSIS OF PROTEINS

Proteins are hydrolyzed to amino acids via the protein derivatives listed in Table 64, page 351. Hydrolysis may be accomplished by boiling the protein with acids or alkalis, or by the action of proteolytic enzymes (Table 17, page 83). These agents hydrate the peptide linkages of proteins and peptides, and rupture the chains.



Hydrolysis of protein with 20 per cent hydrochloric acid or 35 per cent sulfuric acid for from twelve to twenty-four hours produces chiefly amino acids, prosthetic substances, and ammonia (amide nitrogen). Acid hydrolysis destroys tryptophane, by condensing it with furfural (from the prosthetic carbohydrates) to produce the brown-black mixture known as humin. Hydrolysis of proteins by potassium, sodium, or barium hydroxide is slightly more rapid than acid hydrolysis. Barium hydroxide conserves tryptophane; but the alkalis destroy cystine and hydroxy amino acids, convert arginine into ornithine, cause loss of ammonia, and rapidly racemize all the amino acids and deaminate some of them. Racemization of the amino acid units of peptides by alkali probably involves enolization of the peptide linkage and subsequent reversion to a racemic mixture.



The terminal amino acids are less easily racemized, and they can be recognized by their optical activity.

Enzymatic hydrolysis is much less destructive than hydrolysis by acids or alkalis. It does not cause racemization, but introduces protein enzymes into the hydrolyzates as impurities. Maximal enzymatic hydrolysis is accomplished *in vitro* by digesting a toluene-preserved protein solution with pepsin for several days, and then digesting it with an enzymatic extract of the small intestine. Trypsin may be substituted for the intestinal extract, with only slightly lower yields. About ten days are necessary for maximal enzymatic digestion; the degree of hydrolysis can be ascertained by amino acid determinations. Enzymatic hydrolysis is very rapid, but an equilibrium is established as the hydrolytic products accumulate. Pepsin hydrolysis of protein leads to the formation of proteoses and peptones; only about 25 per cent is completely split to amino acids, and further hydrolysis cannot be achieved by adding fresh pepsin. One third of the peptide linkages of lactalbumin or ovalbumin are split by pepsin; protamines and peptides are not digested by this enzyme, and cystine is not liberated from casein. The presence of dicarboxylic acid and either tyrosine or phenylalanine units in the substrate increases its susceptibility to the action of pepsin. (For the digestibility of scleroproteins, see Table 70, page 390. When wool is very finely ground it becomes digestible by pepsin and trypsin.)

Trypsin sets free considerably more amino acids than does pepsin; also, it acts more rapidly and digests protamines. Reduced native hemoglobin is not digested by trypsin, while oxyhemoglobin and denatured hemoglobin are. Trypsin hydrolyzes the intermediates formed by pepsin digestion (proteoses and peptones), but it does not attack certain peptides and polypeptides. As a rule, arginine, cystine, and tyrosine are liberated faster than glutamic acid, phenylalanine, or proline from proteins. Trypsin activity apparently requires the presence of an arginine or lysine unit in the substrate, whereas chymotrypsin activity is conditioned by the presence of an aromatic amino acid unit in the substrate.

The proteolytic enzymes, or *proteases*, are activated by magnesium and manganese cations, cyanide anions, or sulfhydryl compounds. True *proteinases*, such as bromelain, cathepsins, papain, pepsin, and trypsin, hydrolyze polypeptide chains whose amino and carboxyl radicals are largely masked in peptide and grid linkages. Proteins whose free amino radicals are combined with formaldehyde resist the action of proteinases; for this reason, formaldehyde is used in anatomical preservatives and in the production of toxoids. The proteinases do not hydrolyze methylated or racemized proteins, diketopiperazines, or short peptide chains, unless the latter have two peptide linkages in close proximity. Owing to the restricted action of proteinases on terminal peptide linkages, *peptidases* are necessary for maximal conversion of proteins to amino acids. Because proteinases can attack linkages situated at points remote from the terminations of peptide chains, they have been termed *endopeptidases*, while peptidases which attack only much shorter chains are termed *exopeptidases*.

organisms convert simple nitrogenous compounds and atmospheric nitrogen, respectively, into proteins. The latter are utilized by animals, and the nitrogen is excreted chiefly as urea, uric acid, and ammonia. These excretory products are eventually reconverted to nitrates through bacterial putrefaction. Animals can utilize limited quantities of nitrogen from administered urea and ammonium salts, especially the herbivorous animals, whose gastro-intestinal flora synthesize amino acids. The N^{15} of ingested isotopic ammonia can be detected in the amino radicals of tissue proteins and in the nitrogen bases of the nucleoproteins.

Proteins are necessary in mammalian diets to maintain health, weight, and growth. The average daily protein intake of the human adult is in the neighborhood of 100 gm. It provides approximately 15 per cent of the daily caloric intake. Man's protein intake is influenced by climate and by economic factors; protein foods, and particularly those of animal origin, are relatively expensive. The daily protein intake is approximately 75 gm. in warm countries, 125 gm. in temperate climates, and as much as 500 gm. in polar regions. The approximate protein contents of common foods are given in Table 72; gelatin is the only food which consists chiefly of protein. Foods containing from 15 to 25 per cent protein include cheese, eggs, fish, meat, and nuts. Cereal foods have approximately 10 per cent, while fruits and vegetables contain only small quantities of protein. Animal proteins have more varied assortments of amino acids than plant proteins, and they have higher biological value (the ability to support growth and to maintain health and weight). The proteins of eggs, meat,

TABLE 72

APPROXIMATE PROTEIN CONTENT OF COMMON FOODS¹

	PROTEIN (PER CENT)
Gelatin (solid)	90
Cheese, peanut, sardine, shrimp, turkey	25
Almonds, beef, cottage cheese, chicken, duck, fish, ham, liver, lobster, mutton, pork	20
Crab meat, eggs, English walnuts	15
Bacon, bread (wheat), breakfast cereals (wheat), chocolate, condensed milk, crackers, pecans, zwieback	10
Beans (shelled), cakes, ice cream, oysters, peas, pies, puddings	5
Brussels sprouts, cocoa (beverage), cocoanut	4
Beets, broccoli, corn, cream, macaroni, milk, oatmeal, raisins, rice, sauer- kraut, soups, spaghetti	3
Asparagus, egg plant, potatoes, spinach	2
Beans (string), berries, butter, cabbage, carrots, cauliflower, celery, sugar products, coffee (beverage), fruits, fruit juices, lettuce, lard, olives, pumpkins, salad oils, squash, tea (beverage), tomatoes, turnips	0-1

¹ The values given for cereals and vegetables refer to boiled or cooked foods.

and milk also have a high satiety value. At least 50 gm. of proteins of high biological value should be included in the daily diet. Cooked or denatured proteins are frequently digested more easily than are native proteins.

GASTRIC DIGESTION

The gastric juice of vertebrates contains pepsin and rennin. Inactive pepsinogen is secreted by the gastric mucosa and is converted, in the presence of hydrochloric acid, to the active pepsin of gastric juice (pages 80 and 134). Pepsin hydrolyzes pepsinogen to pepsin and an inhibitor substance. The latter is then destroyed by further hydrolysis. Pepsin has a molecular weight of 38,000; it is an acid protein with 5 free amino and from 50 to 55 free carboxyl radicals. The inhibitor is a basic polypeptide with a molecular weight of approximately 5,000; it has 21 free amino and no free carboxyl radicals. Pepsin is unstable above pH 6, while pepsinogen is stable between pH 6 and 9. Pepsinogens are present, in small quantities, in the gastric mucosa of newborn animals; they are produced in quantity soon after birth.

Pepsin rapidly hydrolyzes most native proteins at pH 2, converting them into primary and secondary proteoses and smaller quantities of metaproteins and peptones. It does not digest keratins, nucleins, or protamines, and it hydrolyzes collagens rather slowly. Pepsin digests raw egg white less readily than denatured or coagulated egg white. After leaving the gastric cavity, pepsin is apparently hydrolyzed by trypsin; only traces of pepsin enter the blood or escape into the feces.

In the gastric juice of suckling mammals there is a different proteinase termed rennin or chymosin; it exists in the infantile mucosa as prorennin. Very small quantities of rennin are secreted by the gastric mucosa of adult mammals. Rennin is a specific phosphoproteinase which hydrolyzes casein to whey albumose and a metaprotein (paracasein). Casein is one of the most easily digested proteins; it constitutes a large fraction of the milk proteins (Table 74, page 410). The formation of paracasein by rennin is the basis for the commercial production of cheese. Paracasein is less soluble than casein, and it combines readily with calcium ions to form a curd or coagulum (calcium paracaseinate). Pepsin and other proteinases can also form paracasein from casein, but rennin is more efficacious. It digests only casein and vitellin; the optimal pH of rennin is 3.5. Pepsin has a lower optimum pH, and it is more easily destroyed by alkali than either rennin or pepsinogen. The paracasein curds produced from human milk are smaller, looser, and more digestible than are those from cow's milk, probably because human milk contains less than one half as much protein and calcium as does cow's milk. The comparatively high calcium and casein and the low lactose content of the latter can be modified for pediatric purposes by diluting the milk with water and adding dextrins,

glucose, or lactose. Heating cow's milk or acidifying it with lactic acid allows the formation of a more digestible curd.

It is evident that the gastric digestion of protein produces only slight hydrolysis, preparatory to rapid digestion in the small intestine. The gastric phase of protein digestion normally occupies from 3 to 4 hours.

INTESTINAL DIGESTION

In the duodenum, the partially digested protein mixture is subjected to the action of trypsin (page 141). Since this very active pancreatic proteinase is secreted as inactive trypsinogen, the pure pancreatic juice has feeble proteolytic activity; it can, however, digest casein and peptones by virtue of its carboxypeptidase content. When the pancreatic juice is mixed with intestinal enterokinase, active trypsin is formed and this enzyme digests rapidly all proteins except collagens, keratins, and native reduced hemoglobins. While trypsin has an optimum pH of 8, it operates very efficiently at the pH of duodenal contents (pH 6 to 7). The proteinase is more active than pepsin; it converts the food proteins to peptides and amino acids. Trypsin can slowly digest native proteins, especially those of meat and milk, but it acts more rapidly on the proteose mixtures which result from peptic digestion.

The pancreatic chymotrypsins share the activities of trypsin and, in addition, they exhibit a stronger rennin effect (page 142). The pancreatic juice also contains protaminase and enzymes for removing amide nitrogen and such prosthetic radicals as nucleic acids, phosphoric acid, polysaccharides, and porphyrins.

Enzyme preparations from the intestinal mucosa and succus entericus are sometimes termed erepsin; they are mixtures of aminopeptidase, dipeptidase, leucylpeptidase, prolidase, and prolinase. These enzymes do not require enterokinase activation. Together with pancreatic carboxypeptidase, they complete the digestion of proteins to amino acids by hydrolyzing the peptide remnants of tryptic digestion. The presence of peptidases in the intestinal mucosa affords effective protection against absorption of polypeptides or peptones.

The putrefactive bacteria of the intestine contain intracellular proteinases, and they also secrete such enzymes. The cleavage of proteins to amino acids by bacteria is largely an extracellular process. Many microorganisms (including *Esch. coli*) require the presence of peptone to induce proteolysis.

ABSORPTION

While peptones and peptides are diffusible substances, the normal digestive process converts proteins almost quantitatively to amino acids. It is, therefore, possible to substitute suitable amino acid mixtures for

dietary protein in the nourishment of animals. The amino acids produced from a meat meal are normally absorbed within from six to nine hours. Absorption is most rapid in the duodenum; glycine and alanine are absorbed rapidly, and the branched chain acids rather slowly.

Absorption is usually completed by the time the intestinal contents reach the ileocecal valve. About 1.3 gm. nitrogen is excreted daily in the feces, 0.75 gm. of which is endogenous in origin. Hence, in the normal person, less than 5 per cent of the nitrogen of foods remains unabsorbed. Abnormalities of intestinal motility and of protein absorption increase the fecal loss of nitrogen. Azotorrhea representing 25 per cent or more of the food nitrogen can result from pancreatic disease. Practically all the protein of eggs, milk, and meat, from 80 to 90 per cent of the protein of refined cereals, nuts, and vegetables, and 75 per cent of the proteins of beans, spinach, and whole cereals are digested and absorbed. The proteins of berries, chocolate, fruits, and turnips are digested very poorly. Because of its antitrypsin content, raw egg white is less digestible than cooked egg white.

Amino acids which pass the ileocecal valve, and those administered in nutrient enemas, are partially absorbed from the large intestine. Here, a fraction of the protein hydrolytic products (amino acids, peptides, peptones, and proteoses) undergoes bacterial putrefaction with the production of aporrhegmas (pages 154 and 363). The degree of intestinal putrefaction depends on the quantity of unabsorbed protein, the duration of fecal retention and the types of micro-organisms present.

Allergic responses of men and animals indicate that very minute quantities of peptones, proteoses, and proteins are absorbed through the intestinal mucosa. Mere contact of specific proteins with the mucous membranes of sensitive individuals can, at times, provoke allergic attacks; and appreciable absorption of partially digested protein may produce toxic disturbances and anaphylactoid symptoms (page 488). Normal men and animals do not show these symptoms even though their postprandial blood occasionally reacts with specific antibodies or precipitins for egg proteins, milk proteins, thyroglobulins, and tissue fibrinogens. Transfusion of such postprandial blood to food-sensitive hosts provokes allergic symptoms. Ordinarily, the quantities of proteins and proteoses absorbed from the intestine are exceedingly minute. Undigested egg white proteins are absorbed rather easily by suckling animals and infants, and by patients with a damaged or abnormally permeable intestinal mucosa.

A digestive leukocytosis occurs after protein ingestion. Leukocytes are positively chemotactic to foreign proteins and polypeptides, and during digestion they are attracted toward the intestinal membrane and into the lumen of the gut. Since these cells do not take an active part in the absorption of amino acids, the digestive leukocytosis may be considered as a passive phenomenon.

TRANSPORTATION OF AMINO ACIDS

Concentrations of nitrogenous constituents in normal fasting blood are listed in Table 73. The non-protein amino acid nitrogen of whole blood, as determined in ordinary tungstic acid filtrates, is 6 ± 1 mg. per cent; only one half of this quantity is found when unclaked blood is used for the analysis. The erythrocytes contain glutathione and other peptide constituents which do not diffuse readily. The amino acid nitrogen of blood increases during absorption of a protein meal; the transport is largely in the plasma. The maximal increase is from 2 to 4 mg. per cent at the fourth hour, and the fasting blood level is re-established by the seventh hour. While the rise in amino acid nitrogen is small, it nevertheless accounts adequately for the transportation of all the amino acids absorbed from the intestine. There is a greatly increased blood flow through the active intestine, and the transported amino acids are rapidly assimilated from the blood by the general tissues. Adrenaline lowers the blood amino acid level slightly; insulin has a similar effect, owing to its stimulation of adrenaline secretion. Prolonged fasting causes the amino acid nitrogen of blood to rise a few milligrams per cent. In general, the blood amino acid level is maintained with little variation by balanced synthesis and catabolism of proteins in the living cells. Less than 5 per cent of the amino acid transport occurs in the chyle, but traces of undigested protein which may pass the intestinal wall are carried principally in the lymph. Normal cerebrospinal fluid contains about 3 mg. per cent of amino acid nitrogen.

TISSUE AMINO ACIDS

Normally, the circulating amino acids are assimilated by tissues almost as rapidly as they enter the blood. Only 12 per cent of intravenously injected alanine remains in the circulation after five minutes, and very little is excreted by the kidneys. The amino acid nitrogen concentration of tissues is usually from five to ten times that of the plasma. The tissue amino acids are maintained in dynamic equilibrium with the cell proteins, owing to the synthetic and hydrolytic activities of cellular proteinases (cathepsins). Amino acids are continually being assimilated and liberated by the tissues. The liver is particularly active in removing the absorbed amino acids from the blood; after a protein meal, the hepatic amino acid nitrogen increases temporarily from a fasting level of approximately 45 mg. per cent to as much as 85 mg. per cent. Later the amino acid level of muscle increases slightly.

TISSUE PROTEINS AND STORAGE

In mammals, there is no permanent storage of amino acids; excess amino acids are retained only temporarily by tissues, prior to their deam-

TABLE 73
NITROGENOUS CONSTITUENTS OF BLOOD AND
CEREBROSPINAL FLUID

	WHOLE BLOOD	CELLS ¹	PLASMA OR SERUM ²	CEREBROSPINAL FLUID
	Per Cent	Per Cent	Per Cent	Mg. Per Cent
Hemoglobin	15.6	34		
Protein			7.0 ± 1 ³	28.0 ± 12
Albumins			4.5 ± 0.5 ⁴	23.0 ± 10
Seroglycoid			0.3	
Crystalbumin			4.2 ± 0.3	
Globulins			2.25 ± 0.75 ⁵	5.0 ± 2
Euglobulin			0.4	
Pseudoglobulin			1.8	
α-Globulin			0.65	
β-Globulin			0.9	
γ-Globulin			0.7	
Fibrinogen			0.3 ± 0.1	
Total N	3.3	5.5	1.3	
	Mg. Per Cent	Mg. Per Cent	Mg. Per Cent	Mg. Per Cent
Non-protein N	30.0 ± 5	39.0	23.0 ± 3	20.0 ± 9
Urea N	15.0 ± 3	15.0 ± 3	15.0	10.0 ± 5
Amino acid N	6.0 ± 1 ⁶	8.0 ± 1 ⁶	4.5 ± 1	2.5 ± 1.5
Creatine ⁷	4.0 ± 1	8.0 ± 2	0	
Uric acid ⁷	3.0 ± 1	2.0 ± 1	4.0 ± 1	0.9 ± 0.6
Creatinine ⁷	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.0 ± 0.5
Residual N	9.0	14.0	4.0	4.0 ± 2.0
Glutathione ⁷	40.0 ± 7	94.0 ± 20		
Ergothionine ⁷	18.0 ± 8	35.0 ± 15	0	
Phenols	1.5 ± 0.5			
Guanidine	0.4 ± 0.1			
Glutamine			8.0 ± 2	
Citrulline			0.65 ± 0.35	
Indican			0.25 ± 0.2	
Bilirubin			0.5 ± 0.3	
Urobilin			0.2	

¹ 45 ± 5 per cent of whole blood.

² 55 ± 5 per cent of whole blood.

³ In the newborn, 5.5 per cent. See page 396 for the composition of the electrophoretic fractions of plasma proteins.

⁴ In the newborn, 3.8 ± 0.3 per cent.

⁵ In unalaked blood, 3 ± 1 mg. per cent.

⁶ In unalaked blood, 1.0 ± 1 mg. per cent.

⁷ Concentrations in whole blood, as mg. per cent nitrogen, are: creatine, 1.3; uric acid, 1.0; creatinine, 0.55; glutathione, 5.1; ergothionine, 3.3.

⁸ In the newborn, 1.7 ± 0.3 per cent.

ination and oxidation or their conversion to protein. Except in eggs and seeds, there is no special reserve form of protein comparable to tissue glycogen or the neutral fat of adipose tissue. All fractions of liver protein are augmented by a high protein diet, and this protoplasmic reserve is removed whenever the protein intake is lowered. Adequate dietary protein is important for hepatic regeneration; optimal protection of the liver against toxic agents is afforded by an adequate caloric intake in the proportion of 20 per cent protein, 75 per cent carbohydrate, and 5 per cent fat (page 560). When protein is fed to animals that have lost weight as the result of protein starvation, the tissue proteins are augmented; and vice versa, during starvation, a fraction of the cellular proteins can be used as a source of energy. These phenomena cannot be interpreted as pure storage mechanisms, any more than can the increase in body protein during growth. Protein reserves created in the body become a part of the protoplasmic machinery; they involve hypertrophy, or increase in cell size. In normal adults dietary nitrogen is not stored; as much nitrogen is eliminated daily as is ingested.

Proteins constitute the major fraction of the organic protoplasmic matrix; some of the cellular proteins are enzymes which direct metabolic processes. Approximate concentrations of proteins in normal tissues are given in Table 74. The protein content of body fluids may be found in Table 12, page 62. For nomenclature and classification of individual tissue proteins, consult Table 70, page 389.

SYNTHESIS OF TISSUE PROTEINS

The catheptic proteinases of tissues can synthesize peptides from amino acids, when reducing activators ($-SH$ compounds and ascorbic acid) are present; they also catalyze the substitution of one *l*-amino acid for another in peptides and in proteins. The synthesis of peptide linkages requires energy (derived from carbohydrate catabolism), and an adequate supply of amino acids. The intracellular protein systems act as templates or patterns to direct the synthesis of specific proteins. Various degenerative conditions in tissues allow the formation of abnormal proteins (such as amyloid, albuminoids, and mucins), which are deposited in the cells and the tissue spaces. The intracellular introduction of a virus protein provides a template for the synthesis of this abnormal infectious protein.

By feeding isotopic amino acids to animals, it has been shown that continual synthesis and hydrolysis of proteins occur in living cells, especially in the liver and in tissues which are undergoing hypertrophy. Only from 10 to 12 per cent of the N^{15} of administered isotopic amino acids are retained in the body as non-protein nitrogen, and from 35 to 45 per cent are excreted within three days. From 45 to 60 per cent of the N^{15} enter the body protein, even though the animal maintains a constant weight. Ingested amino acids thus continually replace those of tissue

TABLE 74

APPROXIMATE PROTEIN CONTENT OF TISSUES

TISSUE	PROTEIN	COMPOSITION OF THE TISSUE PROTEIN
	Gm. per 100 Gm. of Tissue	Per Cent
Connective tissue		
Yellow elastic	40	Elastin, 80; collagen, 4.5, chondromucoid, 1.
White fibrous	35	Collagen, 90; elastin, 5; chondromucoid, 4.
Erythrocytes	38	Hemoglobin, 85; stroma globulin, 15, carbonic anhydrase, 0.5.
Skin	35 ¹	Collagen, 67; keratin, 33. ²
Lens	35	Albuminoid, 50; β -crystallin, 30; α -crystallin, 20; albumin, trace.
Teeth		
Dentin	22	
Enamel	1.2	
Bone	22	Collagen (ossein), 80; ossealbuminoid and osseomucoid, 20.
Muscle.	20	Myosin, 55; globulin X, 20; stroma protein, 12; myogen, 10; myoalbumin, 1; collagen, 1; adolase, 0.7; phosphorylase, 0.3.
Eggs		
Yolk	16	Lecitho-vitellin, 80; livetin, ³ 20; vitellomucoid, trace.
White	11	Ovalbumin, 60; ovomucoid, 14; conalbumin, ⁴ 14; ovomucin, 8; globulins, 4; lysozyme, 2.5.
Liver	15 ⁵	Hepatoglobulins, 90; hepatoalbumin, 10, or less; collagen, 1.
Lung	14	
Brain	8	
Blood plasma	7	Albumins, 67 (crystalbumin, 62; seroglycoid, 5); globulins, 33 (α -globulin, 10; β -globulin, 13; γ -globulin, 10) or (euglobulin, 7; pseudoglobulin, 26); serum mucoid, trace.
Milk		
Cow	3.5	Casein, 80; lactalbumin, 15; lactoglobulin, 5.
Human	1.5	Casein, 50; lactalbumin and lactoglobulin, 50.
Lymph (extremities)	2.5 ⁶	Albumins, 78; globulins, 22.
Synovial fluid	1.3	Albumins, 70; globulins, 17; mucin, 13.
Cerebrospinal fluid.	0.027	Albumins, 82; globulins, 18.

¹ This is the protein content of the dermis; the epidermis has a higher protein content, because of dehydration of the outer layers.

² Mostly keratin in the upper layer, kerato-hyalin in the lower layer.

³ Perhaps identical with the serum globulin of the hen.

⁴ Probably identical with the serum albumin of the hen.

⁵ Protein content more variable than in other mammalian tissues.

⁶ Intestinal lymph contains 5.0 per cent, and hepatic lymph 7.0 per cent protein. (For other body fluids, see Table 12, page 62.)

proteins, without altering the structure or quantity of the proteins. The administration of amino acids which contain deuterium at the α -carbon has shown that 10 per cent of the liver proteins and 2.5 per cent of muscle proteins of animals are regenerated in three days. The half lives of liver and plasma proteins are 7 and 14 days, respectively, according to experiments with amino acids containing N^{15} . Myogen has a high rate of renewal, while myosin and hemoglobin are regenerated more slowly. Protein regeneration is most rapid in the liver and plasma, intermediate in visceral organs, and slowest in muscle and skin. Tissue glutathione is regenerated even more rapidly than proteins; half of the glycine and glutamic acid units of liver glutathione can be replaced in 4 hours.

Normal synthesis of muscle proteins is dependent on maintenance of proper sympathetic innervation. Muscular hypertrophy is stimulated by exercise over a period of weeks. Hyperplasia often accompanies hypertrophy, as has been demonstrated in the pregnant uterus by the administration of colchicine, which arrests mitosis at the prophase. Hypertrophy and hyperplasia of the pregnant uterus are stimulated partly by estrogens and progesterone. Atrophy of the uterus, caused by removal of the ovaries, is reversed by the injection of estrone. Distension undoubtedly contributes to uterine hypertrophy, since the repeated distension of other smooth muscles, and cardiac muscle, produces hypertrophy of these tissues. The intestine, kidneys, and liver undergo hypertrophy on high protein diets, but the behavior of the liver is unique in that its protein concentration increases. All fractions of the hepatic protein participate in the creation of this protein reserve. The somatotrophic or growth hormone of the anterior lobe of the pituitary, testosterone, thyroglobulin, and thyroxine exert marked positive influences on growth and protein synthesis. Thyroxine increases both the protein content and size of the liver. Ascorbic acid stimulates collagen synthesis in connective tissue. Proteoses stimulate growth of cells in tissue cultures; embryo juice is an excellent source of the stimulating proteoses or *trephones*.

Plasma Proteins

The proteins of the blood plasma have considerable clinical significance. In addition to the protein fractions listed in Tables 73 and 74, normal human plasma contains an undetermined quantity of prothrombin. Plasma protein values for the human fetus and infant are lower than those found in the adult, although the fetal serum γ -globulin is high and 20 per cent of the protein in calf serum is a fetal type of globulin (fetuin). The adult levels (7 ± 1.0 per cent total protein, 4.5 ± 0.5 per cent albumin, and 2.25 ± 0.75 per cent globulin) are reached by the age of 18 months. The normal albumin-globulin ratio of blood plasma is approximately 2 ± 0.5 ; in lymph the ratio is near 3 ± 0.5 . The proteins of plasma are combined with lipides and inorganic cations (page 56).

Plasma proteins can be depleted, experimentally, by plasmapheresis (the withdrawal of blood and reinjection of the washed erythrocytes suspended in Ringer's solution). The maximal daily regeneration of plasma protein in normal plasmapheretic dogs and in nephrotic patients is approximately 0.4 g. per kg. of body weight. The normal quantity of circulating plasma protein can be replaced within one week, provided the animal receives sufficient dietary protein. Only one tenth of this amount can be regenerated in a similar period on a protein-free diet. In plasmapheretic dogs with inadequate dietary protein, from 40 to 50 gm. hemoglobin can be regenerated weekly in contrast to 3 or 4 gm. of the plasma protein.

Plasmapheretic studies have shown that the liver is the organ which is primarily concerned in the synthesis of plasma proteins; the intestine may also participate to some extent. In the embryo, the primitive plasma protein is formed by disintegrating angioblasts. Bone marrow apparently does not play a special role in the adult, since the plasma protein level remains normal during aplastic anemia. The lymphocytes are reported to contain some serum γ -globulin. Certain fractions of plasma globulin may originate from tissues other than the liver, but this organ normally produces the plasma albumin, fibrinogen, and prothrombin, as well as the major portion of the globulin. Hepatectomy causes a 30 to 60 per cent reduction in plasma fibrinogen. Liver injury retards protein regeneration and lowers the plasma protein level, whereas injury to other tissues increases the plasma fibrinogen. Protein starvation, malnutrition, infection, kidney disease, and repeated injections of gum acacia or gelatin exert inhibitory effects on the hepatic synthesis of plasma proteins. Prolonged hypoproteinemia *per se* seems to stimulate the synthetic ability in normal dogs. Ingested beef serum proteins are 38 per cent convertible to plasma protein. Other conversion factors are: gizzard, kidney, thyroid, and yeast, about 20 per cent; rice, lactalbumin, muscle, and egg white, 18 per cent; liver and potatoes, 15 per cent; heart, casein, and liver extract, 12 per cent; spleen and erythrocytes, 10 per cent; brain, pancreas, stomach, and salmon flesh, 7 per cent; zein, none. Injected mixtures of essential amino acids, or hydrolyzed casein, stimulate plasma protein synthesis; cystine seems to be particularly effective.

The liver regenerates fibrinogen easily, but serum albumin with difficulty; relatively little albumin is present in the liver or other tissues (Table 74). After hemorrhage, a portion of the serum albumin is restored rapidly during the first day by mobilization of a limited albumin reserve in the tissue fluids. In fasting dogs, the total body reserve of plasma proteins and their precursors available for rapid replacement is approximately equivalent to one half the total circulating serum protein. In normal dogs it is three times this quantity, or the equivalent of all the liver protein. Since only a fraction of the hepatic protein is available, it is evident that some reserve protein is present in other tissues. With continued plasma-

pheresis, the reserve becomes exhausted in a week or two, and the albumin-globulin ratio falls.

Plasma proteins have important embatic functions, and they are largely responsible for the plasma oncotic pressure which assists in the control of water metabolism (page 618). The normal oncotic pressure of human plasma is about 28 ± 2 mm. of mercury. Each per cent of albumin in the plasma exerts an osmotic pressure equivalent to 7.54 cm. of water, or 5.54 mm. of mercury, whereas the osmotic pressure for each per cent of plasma globulin is only 1.95 cm. of water, or 1.43 mm. of mercury. Decreased plasma albumin is, therefore, a powerful factor in the movement of fluid toward the tissues and tissue spaces, and in the production of edema. Preparations of human serum albumin are used in the treatment of burns, edema, and shock; each gram of serum albumin injected intravenously can withdraw about 17.5 ml. of fluid from the tissue spaces into the blood stream.

Plasma proteins are used and resynthesized constantly. They can be converted into other body proteins, as shown by the injection of homologous serum or plasma into fasting animals. Such injections can replace dietary protein effectively, maintain the nitrogen equilibrium, and supply all the nitrogenous substances necessary for maintenance of the animal. Since albumins and globulins are utilized at the same rate, changes in the albumin-globulin ratio of plasma are probably due to disturbances in their regeneration. The increased blood amino acid level and the increased urinary excretion of nitrogen and glucose, which result from feeding plasma protein to phlorhizinized dogs, do not occur if these proteins are injected. Free amino acids are not formed from the injected proteins in appreciable quantities, even though the amino acid units of the plasma proteins can replace those of tissue proteins in a very efficient manner. The mammary gland transforms plasma proteins into milk proteins. With the assistance of leukocytes, tissue cultures can utilize serum proteins; the leukocytes transform the proteins into proteoses, which stimulate cell hypertrophy and growth.

Intravenously administered foreign sera and food proteins are not utilized as readily as are plasma proteins; they are excreted partly in the urine, and they incite anaphylactoid symptoms, toxic albuminuria and a decrease in the serum albumin. Extensive replacement of plasma proteins by physiological sodium chloride solutions during repeated plasmapheresis causes death within several hours. Severe depletion produces fatal shock; and, in dogs, marked edema commences at a plasma protein level of approximately 3 per cent.

A portion of the plasma protein normally penetrates the capillary walls to enter the tissue fluids; this fraction is partially returned to the blood stream by the lymphatic system. Ligation of the thoracic duct causes marked delay in the attainment of a normal plasma protein level following hemorrhage in dogs. About 50 per cent of injected plasma protein labeled

with N^{15} leaves the blood stream of the normal dog within 24 hours. Opposing this transudation is a balancing inflow of plasma protein into the circulation, partly through the lymph. Plasma protein labeled with radioactive sulfur apparently leaves the blood at a normal rate during hemorrhagic shock, but the return flow is subnormal. In the normal human adult, the proteins from a liter of transfused plasma can be transferred readily from the circulation to tissue fluids. The total plasma protein in the blood of an adult man is estimated to be 190 gm., of which serum albumin, serum globulin, and fibrinogen constitute 120, 60, and 10 gm., respectively. About 6 gm. fibrinogen are metabolized and synthesized daily; much larger quantities of this protein are formed during inflammation.

✓ THE ESSENTIAL AMINO ACIDS

Certain tissue proteins can be synthesized from polypeptide fragments of the plasma proteins during protein starvation, or when homologous plasma proteins are injected intravenously. Under normal circumstances, however, the amino acids which have been absorbed from the intestine or hydrolyzed from tissue proteins constitute the principal substrate for protein synthesis. It is now agreed that all but ten of the common amino acids can be synthesized readily by mammals. Those which cannot be synthesized readily are termed essential or indispensable amino acids

TABLE 75

ESSENTIAL OR INDISPENSABLE AMINO ACIDS¹

	REQUIRED IN THE DIET	CAN BE REPLACED BY	
	Per Cent	Enantiomorph	Deaminated Product (α -Keto or α -Hydroxy Acid)
<i>d</i> -Arginine	0.2	—	—
<i>l</i> -Histidine	0.4	Yes	Yes
<i>d</i> -Isoleucine	0.5	No	Yes
<i>l</i> -Leucine	0.9	No	Yes
<i>d</i> -Lysine	1.0	No	No
<i>l</i> -Methionine	0.6	Yes	Yes
<i>l</i> -Phenylalanine . . .	0.7	Yes	Yes
<i>d</i> -Threonine	0.6	No	—
<i>l</i> -Tryptophane	0.2	Yes	Yes
<i>d</i> -Valine	0.7	No	Yes
Total	5.8		

¹ The amino acids listed are essential for mammals. Chicks require in addition either glycine or creatine. Glycine deficiency in chicks causes poor growth, retarded feathering, underdevelopment of muscles, and profound weakness. Combined deficiencies of arginine and glycine result in paralysis, due to spinal cord lesions.

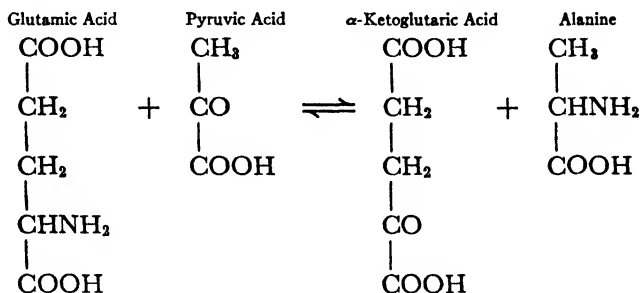
(Table 75). They must be present in the dietary protein in order to allow growth and the maintenance of health, weight, and nitrogen equilibrium. Growth requires a larger intake of essential amino acids than the mere maintenance of body weight and health. Adult human beings do not require histidine for the maintenance of nitrogen balance. When the diet is deficient in an essential amino acid, the animal consumes less food, as though automatically attempting to preserve a normal ratio between the body's supply of amino acids. Thus the deficiency leads to partial starvation; death eventually results if the deficiency is prolonged. Arginine is synthesized more readily than other essential amino acids, but only at a rate insufficient for normal growth; in rats, arginine synthesis allows growth at 70 per cent of the normal rate. Histidine, leucine, and lysine are also more important for growth than for the maintenance of weight. Serious deficiency of lysine produced by feeding deaminized casein to rats causes anemia; in man, lysine deficiency produces nausea, dizziness, auditory hypersensitivity, and increased urinary excretion of organic acids. Tryptophane deficiency in rats is accompanied by cataract, vascularization of the cornea, alopecia, and testicular atrophy; and valine deficiency leads to lack of coordination and extreme sensitivity to touch. Deficiency of phenylalanine in rats causes hypoproteinemia, anemia, narrowing of epiphyseal cartilages of long bones, and atrophy of the adrenal cortex and thymus.

Rats grow well on a mixture of the essential amino acids as the sole source of protein nitrogen. Injection of such mixtures minimizes the emaciation caused by protein starvation. Amino acid mixtures prepared from hydrolyzed casein, and fortified at times with tryptophane, are used therapeutically to stimulate healing of burns, ulcers, and wounds, to relieve symptoms of myotonia atrophica and amyotrophic lateral sclerosis temporarily, to treat hepatic cirrhosis, and to provide parenteral nourishment for surgical and gastro-intestinal patients. The daily intravenous dose of the amino acid mixture is 30 to 120 gm., injected in 2 to 5 percent solution with enough glucose to provide about 1600 Cal. per day. Provision of glucose is important to allow synthesis of protein from the amino acids. The injected amino acids are well utilized, with little urinary loss except when the endogenous catabolism is increased, as by fever. The blood amino acid level returns to the normal value shortly after the injection. Amino acid mixtures can also be given orally.

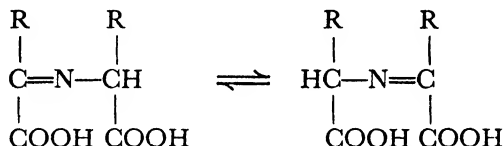
TRANSAMINATION

The deamination products (*i.e.*, the α -keto and α -hydroxy acids listed in Table 67, page 363) can be substituted for the corresponding essential amino acids, except in the case of lysine (Table 75). With this exception, the α -keto acids are readily converted into amino acids in the body. The reaction is endothermic and requires energy from carbohydrate metab-

olism. Certain amino acids and amino acid units of proteins can be converted into others by a shift of the amino radicals to α -keto acids. Thus, brain, heart, kidney, liver and muscle perform the following transamination:



The probable intermediate product is a Schiff's base, which is formed by removal of a molecule of water from the amino acid and the keto acid:

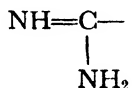


The shift of the amino radical involves mutual oxidation and reduction of the two carbon skeletons. In similar fashion, aspartic acid reacts with pyruvic acid to form oxaloacetic acid and alanine. Only the natural or *l* series of amino acids undergoes biological transamination; the chief keto acids involved are pyruvic, α -ketoglutaric, and oxaloacetic acids. The enzymes which catalyze transamination are termed *aminopherases* or *transaminases*. Dipeptides can serve as substrates for transaminases; in animals, glutathione undergoes transamination more readily than proteins. Transamination is most evident in the proteins of the liver and plasma; transaminase activity is low in hepatoma tissue and in the livers of animals given butter yellow, also in embryonic tissues, pyridoxin-deficient animals, yeast, and *Esch. coli*.

When an isotopic amino acid is fed to an animal, some of the N^{15} is transferred rapidly to amino acid units of tissue proteins. From 12 to 20 per cent of administered isotopic glycine, leucine, and tyrosine enter the tissue proteins in unaltered form, while from 30 to 40 per cent of the N^{15} is exchanged with the nitrogen of other amino acid units. Transamination is particularly active between alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, proline and tyrosine units; it involves the rupture of a single peptide linkage, without separation of the amino acid from the polypeptide chain. Isotopic ammonia and the deuterium of

heavy water are transferred to the α -carbon atoms of amino acid units of proteins.

Lysine does not undergo transamination; this essential amino acid can be degraded in the body, but it cannot be regenerated from its deamination products. Ornithine, a homologue of lysine, is also inactive in transamination; the isotopic nitrogen transferred to arginine is found in the amidine,



nucleus, not in the ornithine portion. Arginine is formed from ornithine through the urea cycle (page 372); and, in mice, administered deuterio ornithine is transformed rapidly to deuterio arginine units of the tissue proteins.

Transamination is largely responsible for the fixing of dietary nitrogen. In the rat's body, about one half of all the protein peptide linkages undergo this change within three days. The dicarboxylic acids, particularly glutamic acid, are most active in this respect; they accept more isotopic nitrogen than do other amino acid units. The average tissue protein contains about 20 per cent dicarboxylic acids (Table 66, page 355). In the entire rat carcass, glutamic and aspartic acids constitute 13 and 12 per cent, respectively, of the total amino acid units.

d-Forms of several essential amino acids can be substituted for the natural *l*-acids, as indicated in Table 75. Considerable *d*-histidine is required, and the enantiomorphs of five of the essential amino acids are not well utilized. The *d*-forms which can be substituted biologically are first deaminated in the tissues to the α -keto acids, which are then reduced and reaminated asymmetrically to form the natural enantiomorphs. Kidney and liver slices, for example, can convert *d*-tryptophane to *l*-tryptophane.

SYNTHESIS OF AMINO ACIDS

Certain amino acids are indispensable because their particular carbon skeletons cannot be synthesized by mammals. Carbohydrate intermediates are important substrates for amino acid synthesis in plants and animals. As a class, the essential amino acids are not very actively gluconeogenic (Table 40, page 226), nor are their carbon skeletons synthesized readily from carbohydrate. Arginine is an exception; it can be synthesized from ornithine by mammals, and it is gluconeogenic. Citrulline and ornithine are undoubtedly the substrates for arginine synthesis. In chicks, citrulline can replace arginine as an essential amino acid. Birds can synthesize considerable ornithine, as shown by formation of ornithuric acid during the detoxication of large quantities of administered benzoic acid. *l*-Cystine is gluconeogenic and is not essential; but it exerts a sparing effect

upon methionine catabolism. When cystine is added to diets containing suboptimal quantities of methionine, it improves growth.

Direct evidence of considerable glycine synthesis in mammals is obtained by feeding large doses of benzoic acid, which is detoxicated by glycine and excreted as hippuric acid in the urine. The amino radicals necessary for glycine synthesis are derived from amino acids which are ordinarily catabolized to urea. Glycine is not formed from administered urea, nor is administered isotopic glycine an important precursor of hippuric acid. Chicks can form glycine from acetate, but only at a rate which is insufficient for normal growth. The calculated maximal rate of glycine synthesis in man is 9 mg. per kg. per hour.

Alanine is synthesized in the mammalian liver from perfused ammonium pyruvate. The synthesis of serine is closely related to that of alanine, and the two amino acids are probably interconvertible in animals. Experiments with N^{15} show that serine can be formed by the carboxylation of ethanolamine. Fungi and *Esch. coli* synthesize tryptophane from serine and indole. The liver can form phenylalanine, tyrosine, and other amino acids from the ammonium salts of the corresponding α -keto acids. This organ will even convert unnatural keto acids to foreign amino acids, which are excreted in the urine.

Dicarboxylic amino acids are synthesized readily in the body and converted to acid amides. In both plants and animals, the dicarboxylic amino acids are chiefly responsible for anchoring the food nitrogen. Plant tissues synthesize asparagine and glutamine *de novo* from ammonia and carbohydrate intermediates, whereas the kidney, retina, and brain synthesize glutamine from glutamic acid and ammonia, by means of the enzyme glutaminase and energy derived from carbohydrate oxidation. That humans can readily synthesize glutamine from catabolic nitrogenous sources has been demonstrated by the detoxication of administered phenylacetic acid. Administration of heavy water demonstrates that the glutamic acid units of tissue proteins are largely renewed each day.

Certain amino acids can be formed from others by oxidation in the liver, kidney and intestinal mucosa. Experiments with deuterio isotopes have confirmed the conversion of ornithine to proline and glutamic acid, the oxidation of hydroxyproline and proline to glutamic acid by kidney tissue, and the partial oxidation of phenylalanine to tyrosine in the liver. Patients with the rare abnormality of protein metabolism known as tyrosinosis excrete tyrosine and its deamination product, *p*-hydroxyphenylpyruvic acid, in the urine. The administration of phenylalanine to such patients causes an increased elimination of both excretory products.

Diiodotyrosine and thyroxine can be formed from the tyrosine units of proteins *in vitro*, and also in various mammalian tissues. However, the thyroid gland produces iodo amino acids much more rapidly than do other tissues. Studies with thyroid slices and radioactive iodide indicate that the synthesis of iodo amino acids is linked to aerobic oxidation; it is inhibited

by thiouracil, thiourea, thiocyanate, sulfonamides, *p*-amino aromatic acids, sulfide, cyanide, azide, and carbon monoxide.

Cystine cannot be synthesized from catabolic nitrogenous sources; its sulfur atom is derived from methionine, and its carbon skeleton from serine, as proved by the aid of C^{13} , N^{15} , and S^{35} isotopes. Formation of cystine from administered methionine is evidenced by the excretion of additional cystine in cystinuric patients, the detoxication of bromobenzene in animals, and transformation into cystine units of the tissue proteins. Cystine is also formed by animals from administered homocystine, and this substance is believed to be an intermediate in the conversion of methionine to cystine; the sulfhydryl radical of homocysteine is evidently exchanged for the hydroxyl radical of serine. The methyl radical liberated from methionine can be used to form choline and creatine, as proved by deuterium labeling. Either *d*- or *l*-homocystine can be substituted for methionine as essential amino acid. Experiments with deuterio derivatives show that the methyl radicals of choline and betaine can be used for the conversion of homocystine to methionine. A large portion of the cystine of ordinary diets is used by animals for keratin formation. The formation of keratin is inhibited on low cystine and low methionine diets, when these amino acids are required for the synthesis of essential tissue proteins. The sulfur amino acids are used directly as substrates for tissue protein synthesis and the sulfhydryl radical of cysteine also activates proteinases and stimulates cell division.

BIOLOGICAL VALUE OF PROTEINS

The varied concentrations of essential amino acids in individual proteins produce marked differences in nutritional value. Proteins which do not contain all essential amino acids in the necessary concentrations cannot serve as the sole sources of nitrogen for mammals. Owing to their full complement of essential amino acids, the proteins of eggs, meat, and milk have superior biological value; but certain plant proteins are deficient in this respect (Table 66, page 355). Gliadin, of cereals, has a low lysine and tryptophane content, and it will not support normal growth. Similarly, amandin, of almonds, is deficient in lysine and valine; and hordein, of barley, lacks lysine, tryptophane, and valine. Zein, of corn, is deficient in lysine, histidine, and tryptophane, and gelatin and arachin (of peanuts) lack sufficient methionine and tryptophane. These proteins cannot maintain body weight. As much as 60 per cent of the nitrogen of vegetable proteins may be wasted in the selection of the proper amino acid assortment for synthesis of tissue proteins. Cereal proteins provide a better assortment, but they are definitely inferior to meat, milk, plasma, and egg proteins. The biological or tissue-replacement values of dietary proteins, as per cent of the total protein, are approximately as follows: eggs, 95; milk, 85; meats and edible mushrooms, 75; cereals and potatoes, 65; nuts, 55; beans, 40; and chocolate, 35. The several food proteins tend to supple-

ment each other; mixed diets of animal and vegetable origin are more efficient than purely vegetable diets. The incorporation of proteins of high biological value into the diet is particularly important for growing children, and for convalescing patients.

ENDOGENOUS CATABOLISM

It has been stated previously that intracellular proteinases operate continually to synthesize and hydrolyze tissue proteins in living organisms. Whether the preponderating activity of an endocellular proteinase is synthesis, hydrolysis, or substitution of amino acid units in proteins depends on the pH, temperature, oxygen tension, energy sources, and the nature and quantities of the coenzymes and available substrates. Both the hydrolytic and synthetic activities of tissue cathepsins are activated by sulfhydryl compounds. In normal adults, the ingested amino acids replace similar units removed from the protein molecules, and the total quantity of body protein tends to remain constant. When hydrolysis preponderates, the cells decrease in size and atrophy results. The tissue protein catabolism which is independent of absorption and transportation of exogenous amino acids is termed the endogenous protein catabolism. Endogenous catabolism can be estimated roughly from the excretion of certain metabolites, namely, creatinine, neutral sulfur, and urochrome. A certain fraction of the excreted uric acid and urea is endogenous in origin. The normal excretion of endogenous metabolites indicates that less than 0.2 per cent of the 11 kg. of protein in a man's body is hydrolyzed daily.

With an adequate caloric intake, muscular exercise does not increase protein breakdown appreciably; but excess thyroxine is a very active stimulant of protein catabolism. When either the protein or caloric intake is inadequate, catabolism preponderates over synthesis and the liberated amino acids are oxidized. During complete starvation, endogenous protein catabolism may increase until the third day; then it gradually diminishes. Two days of starvation cause a 20 per cent depletion of protein in the rat liver but only 4 per cent in other organs. Of the total protein catabolized during prolonged starvation, 16 per cent is derived from the liver, and 62 per cent from skin and muscles. Inactive muscles lose more protein than active ones. Weight losses of the organs of starving animals are recorded in Table 43, page 249, and protein losses in Table 76. Adults who have lost as much as 40 per cent of their body weight can still recover if an adequate diet is provided, but protein starvation in children has more serious consequences.

The chief effect of starvation is atrophy, not hypoplasia. The amino acids liberated from less vital tissues are utilized partly by the heart, brain, and other organs to maintain their protein equilibrium. When salmon return from the sea to fresh water, they do not consume food; large quantities of their muscle proteins are fragmented and transformed into the

TABLE 76

APPROXIMATE LOSS OF TISSUE PROTEINS DURING
ONE WEEK OF FASTING

(In the Rat)

	PER CENT
Liver	40
Gastro-intestinal tract	30
Blood, heart, kidneys	20
Bone, muscles, skin	10
Brain	5
Adrenal glands, eyes, testes	0

proteins of the rapidly enlarging gonads. Following fasting, an adequate diet produces active hypertrophy of the wasted tissues. Carbohydrate food has a protein-sparing action, and it minimizes the endogenous catabolism of tissue proteins. This effect may be attributed to the energy value of carbohydrate and to the use of sugar intermediates (keto acids) in the resynthesis of amino acids and proteins. The smallest excretion of nitrogen (about 2 gm. daily for human beings), therefore, occurs not during complete starvation but on a low protein, high carbohydrate diet. The sparing action which carbohydrate exerts on amino acid catabolism in bacteria is partly due to provision of readily utilizable food for growth and multiplication. As a result, ammonia, which is the principal protein metabolite and nitrogenous food for micro-organisms, is diverted to anabolic processes.

AUTOLYSIS

Under abnormal or toxic conditions, cells can be disintegrated by an unbalanced hydrolytic action of their specific catheptic enzymes. The final postmortem liquefaction of cells has been termed autolysis. Glandular tissues autolyze more rapidly than connective tissues. Collagen is hydrolyzed by cathepsins with great difficulty. Those organs which normally regenerate rapidly contain greater concentrations of proteolytic enzymes, and they autolyze most readily; the brain has a very low concentration of proteinases and it autolyzes slowly.

The optimum pH for autolysis is near the average isoelectric point of the tissue proteins (pH 4 to 6). Local acidity, resulting from asphyxiation, induces rapid postmortem hydrolysis of tissue protein. However, *in vivo* autolysis is probably facilitated less by pH changes than by the inhibition of oxidation reactions which provide the necessary energy transfer for the counterbalancing synthetic processes. Addition of cysteine increases the rate of liver autolysis. During autolysis, the tissue proteins are digested to proteoses, peptides, and amino acids; there is also an accompanying deamination of purines and amino acids, as well as hydrolysis of nucleic acids, lipides, and glycogen by various endocellular enzymes.

Histologically, autolysis signifies extensive disintegration and liquefaction of cells in necrotic tissue; but we may apply the term in a metabolic sense to the removal of cells during physiological regressions of the surface layers of the skin and mucosa, the regressing mammary gland, the ovarian corpora albicans, fetal absorption and the normal disposal of erythrocytes, reticulo-endothelial cells, and so forth. *Fetal autolysis* is common in polytocous animals; the autolytic process commences in the embryonic liver and it is more complete the younger the embryo. Such fetal absorption is stimulated by lactation and by maternal deficiencies of vitamins, or of essential amino acids. *Atrophy* is a type of tissue wasting in which the cytoplasmic proteins decrease in quantity. This phenomenon occurs in inactive muscles, in the tissues of aging animals, during starvation, in certain mental diseases, hyperthyroidism, Simmonds' disease, cancer, cachexia, infections, and so forth. Atrophy is accompanied by a decreased blood supply to the affected tissue, but the process is not always due to this cause. Pathological conditions which produce prolonged hyperactivity of glands or muscles may lead to *exhaustion atrophy* of the tissues concerned.

Prolonged wasting processes lead to degeneration and the appearance of certain pathological proteins. *Hyaline* is a mixture of partially altered cell materials derived from necrotic cells. *Amyloid* is a chondroprotein which infiltrates the tissue interspaces in pathological conditions. It is stained brown by dilute iodine, blue by iodine in the presence of strong sulfuric acid. Massive injections of foreign proteins can induce amyloid formation (page 457).

NITROGEN EQUILIBRIUM

The amino acid digestion products of dietary protein have both an anabolic and catabolic fate. They are used for the synthesis of tissue proteins, hormones, conjugated bile acids, purines, pigments, and methylated derivatives. They can also be deaminated and transformed to carbohydrate and lipid intermediates, or oxidized to provide energy. Comparison of the nitrogen elimination with the protein intake provides an index to the protein balance of the organism. Balance studies must be continued for approximately three days, owing to a lag in the excretion of the nitrogenous metabolites. Such studies demonstrate that the healthy adult is in a state of nitrogenous equilibrium, in which the nitrogen excretion equals the intake. This is a necessary corollary to the fact that protein is not stored readily. Whenever endogenous catabolic processes preponderate, the excretion exceeds the intake and a negative nitrogen balance ensues. Marked nitrogen imbalance occurs in starvation, dehydration, shock, fractures of large bones, fevers, hyperthyroidism, severe acidosis, diabetes mellitus, malnutrition, and wasting diseases. The ingestion of sufficient carbohydrate and the administration of insulin exercise a protein-sparing

action, which aids in adjusting negative nitrogen balances. Growing or convalescing animals have positive nitrogen balances (excess intake over excretion), because of an acceleration of the anabolic or synthetic processes.

The quantity of dietary protein of high biological value necessary for the maintenance of nitrogen equilibrium in normal men is estimated to be approximately 0.7 gm. per kg. of body weight per day. This is equivalent to 50 gm. protein daily for the human adult. Since incomplete plant proteins constitute a portion of the average diet, the recommended daily intake of mixed protein is 1 gm. per kg. of body weight, or 12 per cent of the caloric intake. The protein requirement is nearer 3 gm. per kg. for young children. In late pregnancy, it is about 1.5 gm. per kg. Since the nursing mother secretes from 7 to 15 gm. of milk protein daily, sufficient extra dietary protein must be provided during lactation. With a sufficient carbohydrate intake, protein administration can be reduced temporarily to as little as 0.33 gm. per kg. Nitrogen equilibrium is maintained on high protein diets; but the administration of a great excess in small frequent meals causes a loss of body weight and a negative nitrogen balance, because of exaggerated specific dynamic action, exhaustion of thiamin, and so forth.

DECARBOXYLATION OF AMINO ACIDS

Mammalian tissues can decarboxylate certain amino acids, although normally they produce only very small quantities of amines. Histidine is decarboxylated to *histamine* in all tissues; but the intestinal and gastric mucosae, skin, liver, lung, kidney and muscle perform this reaction most readily. Histidine decarboxylase is inhibited by cyanide, adrenaline, dopa, hydroxylamine, and semicarbazide. Gastro-intestinal tissues liberate histamine-like substances during digestion (page 137); and sunburn and allergic injuries of skin cause the formation of histamine-like H substance in this tissue. The general tissues form histamine during anaphylactic shock. The anaphylactoid symptoms produced by sudden release or injection of histamine are discussed on page 488. Certain of the histidine units of tissue proteins are quite reactive. They are believed to be the source of histamine. The amine is destroyed by the hepatic enzyme, histidase, and more specifically by diaminoxidase (histaminase); histidase ruptures the imidazole ring, while diaminoxidase catalyzes oxidation of histamine to the corresponding aldehyde. Histidase can operate anaerobically, but diaminoxidase is an aerobic enzyme which is inhibited by cyanides, sulfhydryl compounds, ascorbic acid, calcium ion, choline, guanidine, ephedrine, and thiamin. The placenta, pancreas, prostate, adrenal, intestine, kidney, and liver exhibit greater diaminoxidase activity than other tissues. Normal blood contains from 10 to 20 γ per cent of histamine, and lungs have approximately 2 mg. per cent. Small quantities of hista-

mine are excreted in human feces. The ingestion of considerable quantities of histamine has little physiological effect, because the amine is destroyed by intestinal diaminoxidase. For the same reason, the histamine produced by intestinal bacteria has little pathological significance.

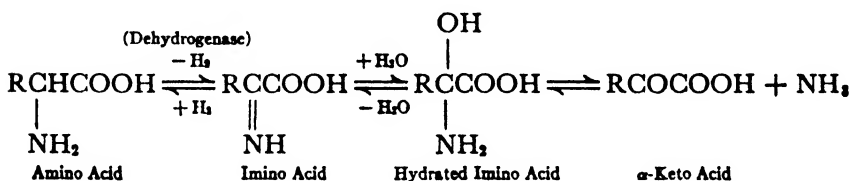
Tyramine is a decarboxylation product formed from tyrosine by the kidney when its oxygen supply is decreased. The tyrosine decarboxylase is present in the distal renal tubules; its activity is accelerated by pyridoxal. The kidney and liver can also form hydroxytyramine by the anaerobic decarboxylation of 3,4-dihydroxyphenylalanine (dopa) through the action of dopa decarboxylase (inhibited by cyanide). Both tyramine and hydroxytyramine are pressor substances. Tyramine is rapidly destroyed in the liver; pressor amines are also destroyed by amine oxidase in the distal tubules of the normal kidney, but not by ischemic kidneys. These amines can be inactivated by quinones. Oxidation and methylation of tyramine in the adrenal medulla is a possible method of synthesis of the hormone, *l-adrenaline*. Normal plasma contains about 0.1 γ per cent of adrenaline, and the adrenal glands contain 100 mg. per cent. This hormone has a powerful vasoconstrictor action, which is utilized in the treatment of hypersensitive states, the bleeding of minor surgery, heart failure, and so forth. Adrenaline has more than twenty times the pressor activity of tyramine, but its effects are temporary, owing to excretion of the hormone in the urine and its rapid oxidation by amine oxidase and other enzymes. Subcutaneously injected adrenaline disappears from the blood within 20 minutes. Adrenaline is closely related to the hormone *sympathin* which is liberated at the endings of most postganglionic sympathetic fibers. The drugs, ephedrine and benzedrine, cause sympathetic stimulation by inhibiting the activity of the amine oxidase which ordinarily destroys sympathin, adrenaline, tyramine, and hydroxytyramine in tissues; ephedrine also inhibits diamine oxidase. The pressor action of ephedrine is more prolonged than that of adrenaline, but it is only 1/100 as intense. Large doses of ergotoxine or ergotamine block the excitatory action of adrenaline.

l-Cysteic acid is decarboxylated to taurine by the liver and kidney; the cysteic decarboxylase is inhibited by cyanide. The muscle dipeptide, *l-carnosine*, contains a β -alanine unit which is the decarboxylation product of aspartic acid. Serine has been shown by the aid of N^{15} to be decarboxylated to ethanolamine in animals. Spermine and spermidine may be formed by decarboxylation of unidentified amino acids. Cadaverine and putrescine are excreted in the urine during cholera and cystinuria. They are formed by the action of lysine and ornithine decarboxylases. The brain contains an oxidative deaminase which is specific for aliphatic amines. The decarboxylases for arginine, glutamic acid, lysine, ornithine, and tyrosine require a coenzyme, which is apparently pyridoxal phosphate ester.

DEAMINATION

The first stage in the oxidative catabolism of amino acids is deamination by α -oxidation. In this process, the amino radical is removed and the corresponding α -keto acid is formed. The α -keto acids can be partially reduced to α -hydroxy acids and fatty acid derivatives. Thus, *Esch. coli* forms indolepropionic acid from tryptophane; certain bacteria produce small amounts of unsaturated urocanic acid from histidine; and aspartic acid is deaminated to fumaric acid by the enzyme, aspartase. That the α -keto acid is the primary deamination product of tyrosine has been demonstrated in alcaptonuric subjects, who excrete homogentisic acid derived from tyrosine catabolism. When *p*-hydroxyphenylpyruvic acid is administered to these individuals they oxidize it to homogentisic acid, and excrete the latter in the urine. The corresponding hydroxy derivative, *p*-hydroxyphenyllactic acid, does not undergo this transformation. It has also been demonstrated that the isolated liver forms pyruvic acid from alanine, and that it subsequently reduces the pyruvic acid to lactic acid.

Deamination of amino acids is catalyzed by amino acid dehydrogenases in the presence of cozymases and flavoproteins (yellow enzymes), which act as hydrogen acceptors. The *l*-amino acid oxidase of liver and kidney is a flavoprotein, which catalyzes the oxidation of the natural amino acids to the corresponding α -keto acids, ammonia, and hydrogen peroxide. It is inhibited by ammonium and cupric cations, iodoacetate, and benzoate. Oxidative deamination probably involves the formation of intermediate imino and hydrated imino acids:



This reaction is reversed during the synthesis of amino acids. Bacterial deamination of amino acids is inhibited by glucose. Deamination liberates only a small quantity of energy; much more is produced by subsequent oxidation of the α -keto acids. However, proteolytic anaerobic bacteria can secure their energy almost entirely by mutual oxidation and reduction of amino acids, in which process alanine, leucine, phenylalanine, and valine act as hydrogen donors, while arginine, glycine, hydroxyproline, ornithine, and proline act as hydrogen acceptors.

The liver and kidney readily deaminate amino acids, and the intestinal mucosa does so more slowly; other mammalian tissues, as well as hepa-

tomas and fetal liver, exhibit little or no oxidative deamination. Brain and retina, for example, can oxidatively deaminate only glutamic acid and glutamine by means of a specific glutamic dehydrogenase; muscle deaminates only aspartic and glutamic acids. The liver actively converts the liberated amino radicals to urea, while the kidney eliminates them as ammonium salts. Hence, the ammonia content of systemic blood is less than 0.1 mg. per cent, and this small quantity is derived chiefly from the deamination of purines (adenylic acid, adenosine) in the general tissues. Purine deamination accounts for only a few per cent of the total deamination in the body. The ammonia content of portal blood increases to 0.3 mg. per cent or more after a protein meal. This ammonia is largely of intestinal bacterial origin, but some results from the action of a urease present in gastric juice, from the hydrolysis of amide nitrogen of proteins by an enzyme of the pancreatic juice, and from slight deamination of amino acids by the intestinal mucosa. In hepatectomized animals, the ammonia of the systemic blood may increase to 1 mg. per cent; removal of the kidney causes very little elevation of blood ammonia. The kidney and liver contain two enzyme systems which deaminate natural *l*- and unnatural *d*-amino acids, respectively. The activity of *d*-amino acid oxidase (a flavoprotein) is lowered in these tissues during riboflavin deficiency; normally it is less in herbivorous than in carnivorous animals, and it is markedly low in hepatoma tissue. Deamination of the unnatural isomers of arginine, aspartic acid, histidine, lysine, serine, threonine, and tryptophane is relatively slow. Adrenalectomy reduces the rate of deamination by the kidney. Glycine is usually deaminated less readily than other amino acids, and histidine and tryptophane are deaminated slowly by the kidney. Glycine oxidase, of the liver and kidney, is a flavoprotein which oxidizes glycine to ammonia and glyoxylic acid, and sarcosine to methylamine and glyoxylic acid. Some of the urinary oxalic acid may arise from glycine by way of glyoxylic acid.

Histidine, histamine, and carnosine can be deaminated anaerobically by the hepatic enzyme, histidase, which ruptures the imidazole ring and produces ammonia from the nitrogen of the ring. It is this imidazole nitrogen, not the α -amino radical of histidine, which reacts with formaldehyde *in vitro*. Histidase converts histidine to two mols of ammonia and one mol of glutamic acid. Hepatomas exhibit low histidase activity. The pyrrolidine rings of proline and hydroxyproline are ruptured by tissues, in somewhat similar fashion, to form glutamic acid. Hydroxyproline catabolism can also follow an alternate course, inasmuch as liver slices transform it partially to acetoacetic acid. Extracts of kidney, brain, retina, liver, and pancreas contain an enzyme, glutaminase, which hydrolyzes the amide radical from glutamine. The kidney produces about 60 per cent of the urinary ammonia from glutamine, and the remainder from amino acids.

CONVERSION OF DEAMINATED RESIDUES TO CARBOHYDRATE AND FAT

Ketogenic and gluconeogenic amino acids are classified in Table 40, page 226. Lysine and tryptophane do not belong definitely to either group. Also, norleucine and hydroxyproline can form both glycogen and acetoacetic acid in rats. The liver is the organ which is chiefly responsible for gluconeogenesis and ketogenesis.

The gluconeogenic values of dietary proteins have been studied in diabetic and phlorhizinized animals; the values vary with the amino acid distribution (page 329). The intravenous injection of homologous plasma protein into phlorhizinized dogs causes much less gluconeogenesis than ingested plasma protein, indicating that hydrolysis of protein to amino acids precedes gluconeogenesis. Alanine and the dicarboxylic amino acids are sufficiently gluconeogenic to produce significant deposits of hepatic glycogen, when fed to rats. The entire carbonaceous residue of glycine can be converted to glucose via glycolic acid. Deaminated *D*-alanine and *L*-cysteine are also completely converted to glucose, but *L*-alanine is relatively inert. Three carbons of aspartic acid, glutamic acid, valine, arginine, and ornithine are convertible to glucose. Histidine, hydroxyproline, and proline appear to be converted to glutamic acid prior to gluconeogenesis. The keto acids derived from the dicarboxylic amino acids are probably first converted anaerobically to succinic and malic acids. Succinic acid is believed to be the gluconeogenic intermediate formed from arginine and ornithine. Threonine and methionine are potentially gluconeogenic. Cysteine may be transformed to the gluconeogenic amino acid, serine. The relation of the urinary G/N ratio to gluconeogenesis has been discussed on page 329.

Phenylalanine, tyrosine, *p*-hydroxyphenylpyruvic acid, and homogentisic acid cause ketogenesis when they are perfused through the surviving liver. Phenylpyruvic acid is not ketogenic under these circumstances, but it is converted to acetoacetic acid by kidney slices. The α -keto acids derived from the aromatic amino acids undergo α -oxidation with loss of the terminal carbon and rupture of the benzene nucleus. The α - and β -carbon atoms of acetoacetic acid are derived from the side chain, and the third and fourth carbons originate from the benzene ring. The deamination product of leucine, α -keto isocaproic acid, is converted to acetoacetic acid by decarboxylation and oxidative removal of a methyl radical. The synthesis of fat from protein apparently requires both thiamin and pyridoxin.

OXIDATION OF AMINO ACIDS

Deamination is usually the first stage in amino acid oxidation; subsequently, the α -keto acid fragments are oxidized to carbon dioxide, water, phenols, and sulfate. α -Oxidation is considered to be the chief process,

although our knowledge of the intermediate steps is by no means complete. In general, tissues can oxidize the α -keto acids more easily than the α -hydroxy acids. Muscle oxidizes only three amino acids (alanine, aspartic acid, and glutamic acid).

A few amino acids can undergo *preliminary oxidation* prior to deamination. Threonine is supposed to be β -oxidized to the corresponding keto amino acid. Deutero ornithine is deaminated to proline, and the latter can be oxidized, with rupture of its pyrrolidine ring, to form glutamic acid in the kidney and liver. Phenylalanine is deaminated to phenylpyruvic acid in the kidney; but in the liver it is partly oxidized to tyrosine, as has been demonstrated by the excretion of this amino acid in the anomaly known as tyrosinosis (page 462). Tyrosine is oxidized more readily than phenylalanine or phenylpyruvic acid in the liver,¹ and considerable phenylalanine is probably converted to tyrosine prior to oxidative deamination. In the condition known as phenylketonuria (page 461), the excretion of phenylpyruvic acid is increased by ingestion of phenylalanine; but tyrosine administration produces only a slight increase. Phenylalanine is an essential amino acid; it can readily be converted to tyrosine, but the reverse transformation is more difficult.

Tyrosine is oxidized by tyrosinase, without loss of nitrogen, to produce the *melanin pigments*. Tissues of ectodermal origin form melanins from tyrosine, dopa, adrenaline and related precursors. Tyrosinase converts tyrosine to dopa (3,4-dihydroxyphenylalanine). The enzyme, dopase, present in melanoblasts, assists in the transformation of dopa to melanins (page 376). Unless traces of copper salts are present in the diet, melanin formation is inhibited. The sulfonamides, *p*-aminobenzoic acid, thiouracil, cysteine, and ascorbic acid inhibit oxidation of tyrosine by tyrosinase and, therefore, the formation of melanin. Melanin production in the skin, with development of sun tan and freckles, is stimulated by exposure to ultra-violet light, whose wavelength is less than 330 m μ . In xeroderma pigmentosum, a fatal actinic dermatosis of infancy which results in malignancy, the skin is abnormally sensitive to the erythematous effect of sunlight. Hereditary factors, the adrenal cortex, local injury of the skin, and melanomas affect melanin production. Dopase is not present in albinos. Melanins undoubtedly exert an important light-absorbing function in the skin. The melanin of fish and amphibian epidermis is contained within melanophore cells which are expanded by the melanophoric hormone of the intermediate lobe of the pituitary gland.

The normal adult can oxidize a little less than 8 gm. of *tyrosine* daily to carbon dioxide, water, and phenols. In normal catabolism, tyrosine can either be oxidized first to 2,5-dihydroxyphenylalanine and then deaminated, or deaminated to *p*-hydroxyphenylpyruvic acid and then oxidized; the product in either case is 2,5-dihydroxyphenylpyruvic acid. The side

¹ In kidney tissue phenylalanine is oxidized more rapidly than tyrosine.

chain of the latter undergoes α -oxidation, and degradation progresses, via homogentisic and acetoacetic acids, to carbon dioxide and water. The liver forms 2,5-dihydroxyphenylalanine and homogentisic acid from tyrosine and phenylalanine, whereas the skin and adrenal glands normally produce some isomeric 3,4-dihydroxyphenylalanine. Alkaptonuric individuals (and vitamin C-deficient guinea pigs) excrete homogentisic acid, because of an hepatic abnormality which interferes with the oxidation of this intermediate metabolite. In tyrosinosis, the metabolic defect inhibits an earlier stage of tyrosine oxidation. Here, homogentisic acid can be oxidized, but its precursor, *p*-hydroxyphenylpyruvic acid, is reduced to *p*-hydroxyphenyllactic acid instead of being oxidized to homogentisic acid. *p*-Hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids are excreted in the urine of tyrosinosis patients, premature infants and scorbutic guinea pigs. Lack of the customary specific dynamic action of phenylalanine and tyrosine in premature infants is additional evidence of abnormality in their metabolism of these amino acids, which can be corrected by administration of ascorbic acid. In tyrosinosis, ingested tyrosine is excreted partly unchanged; when large amounts of tyrosine are fed, some 3,4-dihydroxyphenylalanine appears in the urine.

The method of oxidation of the α -keto acid produced from *tryptophane* is not clear. Feeding tryptophane causes rabbits to excrete kynurenine, while in other experimental animals (dog, fox, guinea pig, rabbit, rat, and wolf), kynurenic acid appears in the bile and urine. Some regard kynurenine as the precursor of kynurenic acid, but it is not certain whether these substances are intermediates in normal tryptophane catabolism or whether they represent side reactions. Kynurenine is excreted only when relatively large quantities of *l*-tryptophane are administered. Kynurenic acid is formed from tryptophane and from indolepyruvic acid by the dog's liver. When administered to man, kynurenic acid is oxidized; it cannot substitute for *l*-tryptophane as an essential amino acid. Kynurenine is produced by oxidative cleavage of the indole nucleus prior to deamination of the side chain; kynurenic acid formation involves formation of a quinoline ring from the side chain. The hepatic enzyme which oxidizes *l*-tryptophane to *l*-kynurenine is termed tryptophanpyrrolase. Kynurenine can be oxidized by kynureninase to anthranilic acid in the mammalian liver; and in pyridoxin deficiency it is converted to xanthurenic or 4,8-dihydroxy-2-quinoline carboxylic acid, which is excreted in the urine. It has been claimed that tryptophane and kynurenine are precursors of urochrome, a pigment of normal urine. Kynurenine is the v^+ gene hormone (page 496), and it is the precursor of the ommochrome pigments of insects.

The oxidative catabolism of alanine, tyrosine, leucine, phenylalanine, glycine, and histidine causes an increase in the metabolic rate of animals (page 114). This thermogenic effect, termed the *specific dynamic action of protein*, approximates 200 calories daily in the normal adult. The thermo-

genic action has been traced to hepatic catabolism of the amino acids mentioned. Thyroxine and dinitrophenol have a much more pronounced thermogenic activity. The administration of thyroxine stimulates the endogenous catabolism of amino acids. Thyroxine is oxidized slowly by tissues, whereas the iodine of administered diiodotyrosine can be rapidly converted to inorganic iodide in mammals.

PHENOLS

After oxidative deamination of tyrosine and tyramine, the side chains of these aromatic substances can be oxidized partially or totally in the liver to produce *p*-cresol and traces of phenol, catechol, and *p*-hydroxyphenylacetic acid. Also, tryptophane and indolethylamine can be degraded partially to skatole and indole, which are, in turn, oxidized in the liver to the phenols, skatoxyl and indoxyl. A fraction of the urinary phenol is, therefore, endogenous in origin and it accounts for the phenol excretion of the fetus. A larger and more variable fraction of the phenol of urine originates from intestinal putrefaction. *Esch. coli*, for example, produces indole and indoleacetic acid from tryptophane, under aerobic conditions. Human blood contains 1.5 ± 0.5 mg. per cent of phenols, the major portion of which consists of nitrogenous, ether insoluble substances. Conjugation of phenols with sulfate and glycuronic acid occurs readily in the liver, the kidney, and other tissues (pages 331 and 438). Detoxication of phenols by conjugation is so efficient that 1 gm. of indole may be administered to a dog without causing unusual symptoms. When abnormally high concentrations of free phenols are produced in tissues, toxic phenomena appear. Excessive use of tannic acid in the treatment of burns can induce hepatitis and hepatic necrosis.

CONJUGATION

The body destroys foreign substances by oxidation or by reduction. When extensive degradation is impossible, an alcohol, phenol, or acid is formed by partial oxidation. Aromatic compounds are usually oxidized with difficulty unless they contain a 3-carbon side chain with an amino, keto, or hydroxy radical at the α -carbon atom. Even these derivatives are partly converted to phenols. The alcohols and phenols are conjugated with glycuronic or sulfuric acid; the acids are frequently combined in peptide linkage with glycine, ornithine, glutamine, or cysteine; and the amines are acylated. (See page 331 for conjugation with glycuronic acid.)

All vertebrates, except birds, can conjugate benzoic acid with glycine to form hippuric acid; quinic acid and other benzoic acid precursors are also converted to hippuric acid. Nicotinic acid and a number of aromatic acids are similarly detoxified; the nicotinylglycine is termed nicotinuric

acid. When the body stores of glycine precursors are exhausted, the aromatic acids are conjugated with glycuronic acid. Ingested sodium salicylate is excreted chiefly as salicyluric acid (the glycine conjugate), together with smaller quantities of the glycuronide and free salicylate. Ten per cent, or less, of phenylacetic acid is conjugated with glycuronic acid in humans, who possess the unique ability to substitute glutamine for glycine in the detoxication of aromatic acids (especially phenylacetic acid). Only in fowls can ornithine be substituted for glycine; fowls cannot employ other amino acids for conjugation, and they form neither conjugated sulfates nor acetylated compounds. When aromatic hydrocarbons or the halogen derivatives of benzene are fed to mammals, they are ordinarily conjugated with acetylcysteine to form mercapturic acids. This reaction involves substitution of the halogen radical by the —SH radical of cysteine. Animals also use cysteine for the detoxication of iodoacetic acid. When cystine and methionine are removed from the diet, the halogen derivatives of benzene are oxidized to phenols and conjugated with sulfate. Hydroxy compounds are readily conjugated with inorganic sulfate, and the resulting ethereal sulfates are excreted in the urine (page 438).

Acetic acid is employed for the detoxication of substances which have amino radicals; examples of such conjugates include acetylcholine (page 191), the mercapturic acids, acetyl-*p*-aminobenzoate, and the acetylsulfonamides. There is no store of acetic acid in the body; this substance is synthesized, as needed, from acetylphosphate or acetoacetic acid (also from ethyl alcohol, pyruvic acid, alanine or butyric acid, as shown by administration of deuterio compounds). Acetylation of sulfonamides is increased markedly by giving sodium pyruvate, and less effectively by glucose or sodium acetoacetate. Insulin is said to stimulate acetylation, and administration of glycuronic acid inhibits it. Methylation of nitrogenous substances is another, although less important, form of detoxication (page 439).

The formulae of various conjugation products are given on pages 332, 370 and 377. These products are often less toxic than the original foreign substances; they tend to be water soluble and are excreted in the urine and in the bile. The conjugated acids and glycuronides are rather highly dissociated. (Compare benzoic and hippuric acids, also phenylacetic and phenylaceturic acids in Table 1, page 4).

Since the liver is very active in performing the conjugations discussed above, detoxication reactions have been used as tests of hepatic function. In Quick's hippuric acid test, 6 gm. sodium benzoate are given by mouth, and a four hour urine specimen is then collected and its hippuric acid content determined. Normal persons excrete 3 gm. of the acid in the four hour period, but less is excreted in the presence of hepatic or renal disease. Sodium benzoate is injected intravenously in a modification of the test. The kidney tubules can conjugate benzoic acid with glycine, and the intestinal mucosa can form ethereal sulfates. These syntheses require

energy from biological oxidation. Renal tissue contains an enzyme, histozyme, which hydrolyzes hippuric acid to glycine and benzoic acid.

FORMATION AND TRANSPORTATION OF UREA

Bacteria, protozoa, aquatic invertebrates, and teleost fishes excrete some of the ammonia derived from amino acid deamination, but elasmobranch fishes, amphibia, turtles, and mammals convert it into urea. Urea is, therefore, the principal nitrogenous excretory metabolite of mammals. It is formed in the liver by a special hepatic enzyme, arginase, which hydrolyzes arginine (but not phosphoarginine) to urea and ornithine. The hepatic arginase activity is increased by corticotropin, corticosterone, dehydrocorticosterone, and compound E. It is low in adrenalectomized animals, fetal liver, and hepatomas. Arginase is not present in the livers of birds or saurian reptiles, and uric acid is the chief nitrogenous metabolite of these animals, as well as of insects and terrestrial gastropods. The mammalian kidney contains only a small quantity of arginase, while avian kidneys contain considerable quantities of the enzyme. All tissues of elasmobranch fishes contain much arginase, and these animals have kidneys with a special urea-reabsorbing tubular segment; in consequence, their blood urea levels are very high (from 1,500 to 2,000 mg. per cent). The tissues of such animals are adapted to high urea concentrations and their hearts are stimulated by urea. In mammals, urea and ammonium bicarbonate solutions stimulate tissue repair of infected wounds.

The chemical reactions concerned in the hepatic synthesis of urea from ammonia have been given on page 372. Ammonia and carbon dioxide combine with ornithine to produce citrulline; this substance then adds another ammonia radical to form arginine, and the latter is hydrolyzed by arginase to urea and the original ornithine.¹ Experiments on mice with N¹⁵ and C¹¹ compounds demonstrate continuous ornithine-arginine interconversion involving bicarbonate and the arginine units of proteins. The energy required for urea synthesis is evidently provided by the catabolism of carbohydrate, since bicarbonate and lactate buffers accelerate the synthesis, while phosphate buffers and anaerobiosis inhibit it (page 281). The liver is the only mammalian tissue which synthesizes urea *in vitro*. Ornithine and citrulline accelerate urea synthesis by liver slices. The latter form measurable quantities of urea from sarcosine, taurine, asparagine, and all amino acids, except the dicarboxylic acids, cystine, isoleucine, and phenylalanine. Normally, therefore, urea is derived chiefly from the

¹ Citrulline can also be converted to arginine in kidney slices by an aerobic type of oxidative transamination, in which aspartic or glutamic acid is the source of the nitrogen. It has been reported that the mammalian liver can produce some urea from ammonium chloride without the participation of arginase, and that it can convert glutamine to urea without formation of ammonia.

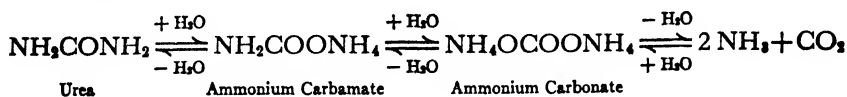
deamination of amino acids in the liver; only traces of ammonia are transported to this organ from the intestine and the muscles. The ornithine cycle of urea synthesis has been demonstrated in fungi (*Neurospora*).

Urea constitutes the major fraction of the non-protein nitrogenous substances of human blood (Table 73, page 408). The non-protein nitrogen and urea levels of blood tend to rise and fall together; they are decreased by diuresis and by low protein intake, and are raised by increased endogenous metabolism, impaired renal function, and dehydration. Urea normally constitutes about 50 per cent of the total non-protein nitrogen of the blood; the remainder represents the nitrogen of amino acids, creatine, creatinine, and uric acid, and the undetermined residual or rest nitrogen. The latter is found chiefly in the erythrocytes; it includes the nitrogen of amines, bile pigments, citrulline, glutamine, hippuric acid, nucleotides, peptides, phenols, thioneine, and so forth. Urea is approximately equally distributed between the plasma and the erythrocytes. It is a very diffusible substance which readily enters all the body fluids. Lymph and transudates contain approximately the same quantity of non-protein nitrogen as does blood plasma, but cerebrospinal fluid has less (Table 73, page 408). Blood urea nitrogen rises to a maximum of from 18 to 25 mg. per cent in about six hours following a protein meal. It is also increased when endogenous catabolism is elevated, or when renal excretion is diminished. The urea nitrogen and non-protein nitrogen of whole blood are important in clinical studies of renal function.

When the liver is removed from a dog, the urea concentrations of the tissues, blood, and urine decrease markedly. If the kidneys and the liver are removed, blood urea is not elevated, but nephrectomy alone causes a marked rise. The liver has an enormous factor of safety for deamination and urea formation; more than 90 per cent of the healthy hepatic tissue must be removed to inhibit these functions markedly, and hepatic disease must be severe before it lowers the blood urea level. Urea formation ceases in hepatectomized mammals, and in terminal atrophy of hepatic glandular epithelium of the human. Under these conditions, urine formation and urea excretion become negligible in from twenty-four to thirty-six hours, indicating that urea is an important normal stimulant of urinary secretion. Urea disappears from the blood, while blood amino acid nitrogen may increase to 200 mg. per cent or more; if kidney function is intact, there is an enhanced excretion of amino acids.

The injection of amino acids or ammonium salts into hepatectomized animals does not produce urea, but the blood ammonia rises slowly from a normal level of less than 0.1 mg. per cent to as much as 1 mg. per cent. This ammonia is derived from intestinal absorption and purine deamination in muscles. Hepatectomized animals are easily poisoned by the administration of ammonium salts. The enzyme, urease, which hydrolyzes urea, is widely distributed in plants, but in mammals it is found only in

the gastric mucosa and in the bacterial flora of the saliva and intestinal fluids. Urease hydrolyzes urea to ammonium carbamate and ammonium carbonate:



Instead of excreting nitrogen, plants store it as asparagine and glutamine which they synthesize from ammonia.

EXCRETION OF NITROGENOUS METABOLITES

Nitrogenous waste products are excreted chiefly by the kidney glomeruli of mammals, and by the proximal renal tubules of certain fishes. Amino acids, creatinine, urea, and uric acid are excreted almost entirely by the kidney glomeruli of man, but small variable fractions of these substances can be eliminated by the tubular epithelium of certain mammals. Urinary nitrogenous metabolites are concentrated during the passage of glomerular urine through the tubules. The average excretion and concentration of nitrogenous substances by the human kidney are given in Table 77.

In elasmobranch fishes, the biliary excretion of urea is much more important than its renal excretion, but the excretion of nitrogenous substances

TABLE 77

DAILY EXCRETION OF NITROGENOUS SUBSTANCES IN NORMAL HUMAN URINE

	GRAMS	GRAMS N	PER CENT OF TOTAL N	APPROXIMATE CON- CENTRATION BY THE KIDNEY
Total nitrogen . . .		12.5 ¹	100	
Urea	22	10.5	85	60
Creatinine	1.3	0.55	4.5	70
Ammonia	0.7	0.57	4.5	
Total amino acids .		0.5	4.0	
Free amino acids . .		0.1	0.8	2
Uric acid	0.7	0.23	2	20
Other purines	0.02			
Hippuric acid	0.7	0.055	0.4	
Imidazole derivatives	0.3			
Phenols	0.2			10
Glycocyamine	0.03	0.01		
Allantoin	0.03	0.01		
Indican	0.01			3

¹ In addition, 1.3 gm. are excreted in the feces and 0.7 gm. by the skin.

in the feces and perspiration of mammals is almost negligible. The fecal nitrogen is mainly endogenous; on an average diet, about 1.3 gm. nitrogen are excreted daily in human feces. The non-protein nitrogen content of normal human perspiration is only 40 mg. per cent, two thirds of which is urea nitrogen. Very small quantities of endogenous nitrogen are lost from the epidermis as keratin, and approximately 0.2 gm. nitrogen is secreted daily in the perspiration at ordinary room temperature; five times this quantity may be lost during profuse sweating. In exfoliating diseases, such as psoriasis, 1 to 2 gm. nitrogen can be lost daily in the scales.

The average daily nitrogen excretion of an adult man is about 14 gm., 12.5 gm. of which appear in the urine. During fasting, the urinary nitrogen excretion falls to about 7 gm. daily; and on a protein-free, carbohydrate diet it may be lowered to 3 or 4 gm. daily. The urinary nitrogen output varies with the protein intake and with endogenous catabolism; it is not increased by muscular exercise. Analytical procedures for the determination of total nitrogen and separate nitrogenous constituents in urine are similar to those for deproteinized blood filtrate. The urine must be preserved, preferably with toluene, and the urea and ammonia analyses should be made early to avoid bacterial conversion of urea to ammonia.

Urea usually accounts for from 80 to 90 per cent of the total urinary nitrogen of the normal adult. The average daily output is 22 gm., or 10.5 gm. of urea nitrogen. When the protein intake is low, the urea nitrogen may constitute only 60 per cent of the total nitrogen. Urea excretion is decreased by low protein diets, myxedema, acidosis (due to ammonia production), severe hepatic disease (due to decreased urea formation), and nephritis (due to lowered excretory ability). It is increased by high protein diets and by exaggerated endogenous catabolism (fevers, diabetes, hyperthyroidism, etc.). Elevation of the blood urea level increases the excretion of this substance by normal kidneys. Urea has a marked diuretic action; the normal kidney excretes the metabolite very efficiently, and blood urea nitrogen levels above 50 mg. per cent are suggestive of renal disease. Determinations of the efficiency of urea excretion and concentration are useful tests of renal function (page 451). About 40 per cent of the urea in the glomerular filtrate is normally reabsorbed by mammalian renal tubules.

The kidney normally secretes 0.7 ± 0.1 gm. ammonia, which is formed by the deamination of glutamine and amino acids in the renal tubular epithelia. It is present in urine chiefly as ammonium acid phosphate, chloride, and sulfate. The relations of urinary ammonia to acidosis have been considered on pages 32 and 37.

The amino acids of the glomerular filtrate are actively reabsorbed, together with water, glucose, and sodium chloride in the tubules. The small quantities found in normal urine therefore represent traces which have escaped reabsorption. The average daily loss is about 0.1 gm. free amino acid nitrogen for the normal adult. In severe liver disease, the

excretion may increase to 1.5 or 2.5 gm. daily, owing to deficient hepatic deamination, and accumulation of amino acids in the blood. Urine also contains about 0.4 gm. nitrogen in the form of combined amino acids, chiefly hippuric acid and sulfur-containing peptides (oxypoteic acids). Excretion of the latter increases in wasting diseases. The average daily excretion of hippuric acid is 0.7 gm. Diets high in fruit and vegetables (especially prunes, cranberries, and plums), which contain benzoic acid or such benzoic acid precursors as quinic acid, can double the hippuric acid output. Herbivorous animals excrete considerable hippuric acid.

Normal human urine contains from 80 to 450 mg. imidazole derivatives daily; less than one half of this mixture consists of histidine. Traces of amines, glycoamine, guanidine, protein, and nitrogenous vitamins are also excreted in the urine. The excretion of creatine and creatinine is discussed on page 442.

Approximately equal quantities of free and conjugated phenols are found in normal urine; the phenolic acids are entirely in the free form. The total excretion of phenolic substances on ordinary mixed diets is approximately equivalent to 200 mg. phenol daily. Included in this mixture are 10 mg. skatole and from 5 to 20 mg. indican (potassium indoxyl sulfate). The feces contain an additional 50 to 60 mg. of indole and skatole daily. Urinary phenol and indican excretion is increased by high protein diets, while starvation, catharsis, and high carbohydrate or milk diets lower the excretion of these substances. The commonly used phosphotungstomolybdate, and diazotized *p*-nitraniline reagents for phenols are non-specific. Determination of that fraction of the urinary phenols which is soluble in ether gives an approximation of the quantity of non-nitrogenous phenols and phenolic acids. These substances constitute only one half the total urinary phenol.

The human kidney begins its excretory functions at the tenth to the twelfth week of embryonic life. The urine of the fetus contains considerable quantities of amino acids and peptides; only 40 per cent of the total nitrogen is present as urea. The chief excretory pathway of the fetus is through the placenta and the maternal kidneys.

SULFUR METABOLISM

Studies with S^{35} isotope prove that plants can use inorganic sulfate for the synthesis of organic sulfur compounds; animals cannot do so. The dietary sulfur compounds required by the mammal include biotin, methionine, and thiamin. Inorganic sulfate is not a dietary essential of mammals, since it is readily formed by oxidation of the sulfur amino acids.

Small concentrations of inorganic sulfate are found in tissues and body fluids. The blood plasma normally contains about 6.5 mg. per cent of non-protein sulfur, which includes 1.3 ± 0.5 mg. per cent derived from inorganic sulfate and 2.7 ± 0.7 mg. per cent each from ethereal sulfate

and neutral sulfur compounds. The sulfates are distributed about equally between plasma and erythrocytes, but the latter contain most of the neutral sulfur (in the form of glutathione and ergothioneine). In the tissues, sulfates are esterified to sulfomucin prosthetic radicals, heparins, conjugated phenols, etc. Such sulfate esters are hydrolyzed by the enzyme, sulfatase. There are exogenous and endogenous phases of sulfur metabolism, and a sulfur equilibrium which is comparable to that of nitrogen. By far the greatest fraction of the sulfur of the animal body is found in organic combination, and the sulfur compounds excreted by the mammal originate largely from protein catabolism. Administration of methionine labeled with S^{35} shows that this amino acid is a precursor of homocystine, cystine, cysteine, and taurine in mammals. The interrelations of the sulfur amino acids have been discussed on page 419. They are used anabolically for the synthesis of proteins, glutathione, and other peptides. The cystine content of cells increases proportionately to the nucleic acid content. Sulfhydryl compounds form important oxidation-reduction systems, which regulate cathepsin activity and nucleic acid synthesis. Some cysteine is partially oxidized to cysteic acid; the latter is decarboxylated in liver and kidney to taurine, which is incorporated into the taurocholic acids. Taurine and cysteic acid are oxidized with difficulty in the body; like inorganic sulfate, they are not convertible to cystine or cysteine. The sulfhydryl radicals of homocystine and cysteine are very readily removed and oxidized to inorganic sulfate, which appears in the urine. The liver contains an enzyme, desulfurase, which converts cysteine to pyruvic acid, ammonia, and hydrogen sulfide. Some methionine is undoubtedly deaminated to the corresponding α -keto acid; details of the sulfur metabolism of this fraction are unknown.

Sulfur compounds are excreted more slowly than nitrogenous metabolites. The ratio of excreted sulfur to nitrogen is usually 1 : 12 in adults, and 1 : 14 in children. Sulfur is excreted principally in the urine, although some is lost in the feces as sulfide, and in the hair and cuticle as keratin. The sulfur metabolites are classified as inorganic, ethereal, and

TABLE 78
DAILY EXCRETION OF SULFUR IN
NORMAL HUMAN URINE

	GRAMS S	PER CENT OF TOTAL S	APPROXIMATE CONCENTRATION BY THE KIDNEY
Total sulfur	1.0	100	
Sulfate	0.88	88	
Inorganic	0.80	80	50
Ethereal	0.08	8	7
Neutral sulfur	0.12	12	3

neutral fractions (Table 78). The total sulfur excretion varies with the protein intake and the endogenous catabolism.

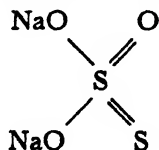
The inorganic sodium, potassium, calcium, and magnesium sulfates of urine are the chief sulfur metabolites; they constitute 90 per cent of the urine sulfate. The daily excretion of inorganic sulfate (Table 78) represents a loss of approximately 550 ml. of N/10 base. The sulfate anion has a diuretic action, but its excretion is relatively difficult; marked sulfate retention may occur in uremia, with elevation of the blood sulfate to as much as 22 mg. per cent (as sulfur).

About 10 per cent of the urine sulfate is ethereal sulfate conjugated with phenols, and so forth. The most important ethereal sulfates of urine are *p*-cresol, phenol, indoxyl, and skatoxyl sulfates. These conjugates are formed principally in the liver; they result from the normal catabolism of aromatic amino acids and detoxication of intestinal aporrhégmas. The excretion of ethereal sulfate is increased by stagnation of intestinal contents, increased protein intake, and bacterial decomposition of tissue proteins (as in gangrene, abscesses, suppuration, etc.).

The neutral sulfur represents a mixture of such organic sulfur compounds as cystine, mercaptans, peptides (oxypoteic acids), sulfides, taurine, thiocyanates, ergothioneine, thiosulfates, urochrome, and the thiazole portion of thiamin. The excretion of neutral sulfur is independent of the protein intake. It is related to endogenous protein catabolism, and is increased in cystinuria, melanuria, and wasting diseases.

Methyl mercaptan and ethyl sulfide have been found in urine; the former causes the peculiar urinary odor following the ingestion of asparagus. As much as 10 per cent of ingested elementary sulfur is converted to hydrogen sulfide in the large intestine; intravenously injected sulfur is similarly reduced in the body. Some sulfide of bacterial origin is normally absorbed from the intestine; it is largely oxidized by tissues to sulfate, but a small fraction is excreted unchanged in the urine. (Sulfites are also rapidly oxidized to sulfate.) Hydrogen sulfide is absorbed by the lungs; in gaseous concentrations below 1 to 2,000 it is rather harmless, because of its rapid oxidation by tissues; but larger concentrations produce hyperpnea, respiratory paralysis, and death.

Normal human blood contains 1.3 mg. per cent of thiocyanate; habitual smokers have higher blood thiocyanate concentrations. In the thiocyanate therapy of hypertension, the blood level should not exceed 12 mg. per cent in order to avoid toxic effects. Thiocyanates are secreted in bile, saliva, and urine; they are formed by the detoxication of small quantities of cyanide from plant foods. There is also a small quantity of thiosulfate,



in mammalian urine; its excretion is increased by feeding cystine and taurine. Thiosulfate, given orally, is absorbed slowly and oxidized largely to sulfate, while one third of that injected intravenously is excreted unchanged. Sodium thiosulfate is administered intravenously in the treatment of heavy metal poisoning and exfoliative dermatitis (especially dermatitis from arsenical drugs). It is also used with sodium nitrite in the treatment of cyanide poisoning.

METHYLATION; METABOLISM OF CREATINE

Nitrogenous substances are methylated readily by plant and animal tissues. Methionine, choline and betaine are methylating agents for the formation of other N-methyl derivatives of animals (adrenaline, alkylamine derivatives, anserine, creatine, creatinine, methylguanidine and sarcosine). Methylation is employed in detoxication of pyridine (to N-methylhydroxypyridinium salts), nicotinamide (to N-methylnicotinamide), and nicotinic acid (to trigonelline). With the exception of acetylcholine, adrenaline, betaine, choline, methionine, and sarcosine, N-methyl and N-acyl amino acid derivatives are relatively stable in the body; when their N^{15} isotopes are ingested, they are partially excreted in unchanged form and partly converted to urea. The interrelations of choline, betaine, and methionine have been discussed on page 235. Rats can demethylate the carcinogen, butter yellow, and hydrolyze its azo linkage.

Dietary *creatine* is derived chiefly from meat and meat products; it is absorbed without change in the small intestine. The fasting blood of normal persons contains about 4 ± 1 mg. per cent, present almost entirely in the erythrocytes (Table 73, page 408). The distribution of creatine in tissues is given in Table 79. Creatine is an important constituent of muscle and nerve tissue. Rapidly contractile muscles contain much more creatine than do smooth muscles. The muscles of an adult man contain approximately 100 gm., the brain, 2 gm., and the liver, 0.5 gm. creatine. In resting muscle 80 per cent (in nerve 45 per cent) of the creatine exists as phosphocreatine united to protein. Hence, muscle creatine does not diffuse readily into tissue fluids. The creatine of the testes is not phosphorylated. Phosphocreatine is essential for rapid muscular contraction, maintenance of muscle tonus, and normal nerve function (page 320). It also exerts a favorable influence on the retention of nitrogen as tissue protein. Limited quantities of creatine are assimilated by normal muscles; 1 gm. ingested creatine is quantitatively retained by the human liver and muscles, but larger amounts are partially excreted in the urine. Women and children retain less creatine than men. Ingested isotopic creatine rapidly replaces the creatine radical of muscle phosphocreatine. Injections of radioactive inorganic phosphate demonstrate continued rapid regeneration of phosphocreatine (78 per cent in three hours).

Creatine is not essential in the diet of mammals; it can be synthesized

TABLE 79

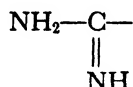
APPROXIMATE CREATINE CONTENT OF TISSUES¹

Muscle	Mg. PER CENT
Striated ²	
Mammalian	450 ± 150
Human	375 ± 25
Cardiac	
Mammalian	215 ± 45
Human	200
Smooth	55 ± 25
Testes	190 ± 90
Brain	100 ± 30
Gray matter	150 ± 30
White matter	70 ± 15
Intestine, kidney	25 ± 5
Liver	20 ± 10
Other glands	15 ± 5

¹ The values quoted for brain, muscle, and testes include about 10 per cent of non-creatine substances. In other tissues this fraction is larger.

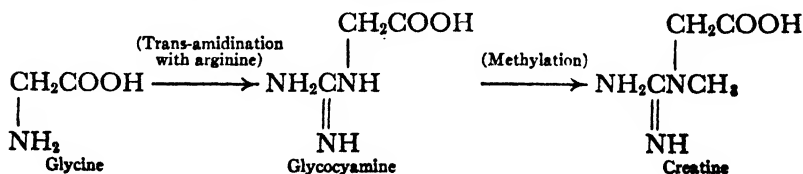
² Red muscle, 350 mg. per cent; white muscle, 450 mg. per cent.

readily in the body from glycine through the intermediate substance, *glycocyamine* (guanidineacetic acid). This intermediate is formed readily by kidney slices, which transfer the amidine radical,



from arginine to glycine. Glycocyamine can also be formed slowly from *sarcosine*, which is first demethylated. Such demethylation has been demonstrated in mammals by the conversion of ingested sarcosine to hippuric acid (also, isotopic sarcosine and betaine to glycine). Sarcosine is not regarded as a normal physiological precursor of creatine, since the N¹⁵ of ingested isotopic sarcosine is found largely in the glycine units of tissue proteins. The N¹⁵ of administered isotopic ammonium salt is transferred to the amidine radical of creatine, but not to sarcosine. Glycocyamine is hydrolyzed to glycine and urea by the hepatic enzyme, *glycocyaminase*. From 25 to 40 mg. glycocyamine are excreted daily in normal human urine.

Creatine is formed from glycocyamine by methylation:



These transformations have been confirmed by feeding isotopic glycocyamine. Methylation of glycocyamine occurs in the liver, and the methylating agent is probably methionine, although betaine and choline are also physiological methyl donors, as shown by experiments with N^{15} isotope. Liver slices form more creatine when methionine is added to the perfusion fluid. Glycine administration increases the muscle creatine of normal animals, and enhances its excretion whenever creatinuria exists. In the chick, glycine is a dietary essential for adequate creatine synthesis; the symptoms of glycine deficiency (page 414), including the subnormal muscle phosphocreatine content, can be prevented by creatine feeding. Administered glycocyamine is five times as effective in stimulating creatine synthesis in mammals as is either glycine or sarcosine. When sufficient glycocyamine is fed to animals on a low methionine diet, they show weight loss and fatty livers, as in methionine deficiency. Creatine formation is independent of the quantity of protein in the diet. Studies with N^{15} compounds show that creatine is regenerated much more slowly than the amino acids. Only 2 per cent of the body creatine is regenerated daily, but this quantity is sufficient to replace that which is excreted in the urine as creatinine. Damage to muscle or nerve tissue causes rapid cleavage of phosphocreatine and liberation of creatine into the tissue fluids. Lowering the glycogen reserve of muscle decreases its ability to retain creatine; hence, creatine appears in adult male urine by the third day of starvation.

Certain bacteria can synthesize *creatinine* from glycine, urea, and glucose. This anhydride is the sole end product of normal mammalian creatine metabolism, as proved by studies with isotopes. In the adult human, about 1.3 per cent of the total body creatine is converted each day to creatinine and excreted in the urine. Muscles and testes contain about 4 mg. per cent, and other tissues less than 1 mg. per cent creatinine. There are 1.5 ± 0.5 mg. per cent in the whole blood, equally distributed between plasma and erythrocytes. By means of specific bacterial adaptive enzyme preparations, the chromogenic substance of the plasma and urine which reacts with alkaline picrate, and about one half that present in the erythrocytes, have been shown to be creatinine. The plasma of uremic patients contains some non-creatinine chromogenic material. The creatinine content of lymph, transudates, exudates, and cerebrospinal fluid is approximately equal to that of plasma. Both creatine and creatinine traverse the placenta readily; fetal and maternal bloods have the same concentrations of these substances. Nephrectomy and severe kidney disease cause a marked increase in blood creatinine and smaller accumulations of blood and liver creatine.

Creatine is probably converted to creatinine in the muscles; the liver plays no special role. An enzyme, creatine anhydrase, which catalyzes the reaction has been detected in certain soil bacteria. The formation of creatinine involves removal of water from creatine and the formation of an intramolecular peptide linkage. Studies with isotopes indicate that

this transformation is irreversible in animals. Prolonged administration of creatine to dogs causes a gradual increase in creatinine excretion and eventual elimination of some creatine in the urine. The normal kidney excretes creatinine even more rapidly than urea. Creatinine is concentrated about seventy times during excretion; unlike creatine and other nitrogenous metabolites, it is not reabsorbed by the renal tubules. Creatinine has a threshold value of approximately 6 or 7 mg. per cent (in whole blood, or 0.58 mg. per cent in plasma); creatinine is a non-threshold substance. When 1.5 gm. creatinine are given by mouth, approximately 75 per cent is normally excreted within six hours, and most of the remainder within twelve hours.

The human adult excretes about 1.3 gm. creatinine daily, independent of muscular exercise or protein intake. Creatinine is, therefore, a typical endogenous metabolite. Since it is a very characteristic constituent of urine, the determination of total creatinine (*i.e.*, creatine plus creatinine) can be used as an index to the accuracy of urine collection. Total creatinine excretion is constant for an individual from hour to hour and day to day. The urinary output is frequently expressed as the *creatinine coefficient*, or the mg. of creatinine per kg. of body weight excreted daily. Average human creatinine coefficients (in terms of creatinine) are as follows: 24 ± 8 for men, 18 ± 9 for women, 12 ± 4 for children, and less than 8 for infants; or, in terms of creatinine nitrogen, 9 ± 3 for men, 6.5 ± 3 for women, 4.5 ± 1.5 for children, and less than 4 for infants. Creatinine coefficients of athletic women approach those of men. When the diet contains neither creatine nor creatinine, the excretion of the latter is proportional to the muscular mass and development.

Less than 20 mg. of creatine is excreted daily by a normal man, whereas the average urinary creatine excretion of women is near 50 mg. daily. Eunuchs, children, infants, pregnant or parturient women, various mammals, ruminants, and birds excrete greater quantities of creatine than do normal human adults. Infants and children apparently form as much creatine per kilogram of body weight as adults do, but they do not retain it completely. The muscles of infants contain only one half the quantity of creatine characteristic of adult muscle. The urinary creatinine/creatinine ratio rises during early life until adult creatinine coefficients are attained at approximately the seventh year. At this age, creatinuria ceases in boys, but girls continue to excrete creatine until puberty, when the creatinuria becomes intermittent with the menstrual cycles. The marked creatinuria which occurs during parturition is related to lactation. Creatinuria is usually at the expense of creatinine excretion; it occurs in both sexes whenever tissue is broken down, as in fevers, starvation, diabetes mellitus, and wasting diseases (page 462). The creatinuria of starvation is related to abnormal carbohydrate metabolism; feeding a protein-free carbohydrate diet inhibits the creatine excretion. Excess thyroxine causes marked lowering of muscle phosphocreatine and pronounced creatinuria; an

exceptionally large proportion of administered creatine tends to be retained in myxedema.

PATHOLOGY

"Life and death seem to have no separate existence in nature; anabolism is unintelligible apart from catabolism; pathology is an integral part of physiology." — MORRIS R. COHEN

HYPOPROTEINEMIA

Prolonged or excessive albuminuria leads to decreased plasma protein levels. The plasma protein is usually normal during acute glomerulonephritis; when hypoproteinemia occurs, the prognosis is unfavorable. The low plasma protein level in chronic stages of nephritis tends to return to normal during the terminal or uremic stage, owing to hemoconcentration and diminished albumin excretion. While albuminuria is the chief cause of the hypoproteinemia of renal disease, there is frequently a decreased ability to regenerate plasma albumin. Continued urinary loss of 4 or 5 gm. protein per day is, at times, accompanied by a gradually developing hypoalbuminemia, even though a normal adult can synthesize 25 to 30 gm. plasma protein daily. Deficient synthesis is an especially prominent feature of lipid nephrosis. In this condition, the plasma albumin may decrease to 0.15 per cent with compensatory rises in plasma fibrinogen and globulin to as much as 1 and 5 per cent, respectively. In nephrosis, the plasma γ -globulin fraction is diminished, and the α - and β -globulin fractions are increased. Hypoproteinemia is a constant feature of chronic nephrosis; plasma levels below 3 per cent protein and 2 per cent albumin are not infrequent. Inadequate protein intake aggravates hypoproteinemia and edema in nephritic, nephrotic, and cardiac patients.

Advanced stages of chronic hepatic diseases are often accompanied by moderate hypoalbuminemia, and by increased β - and γ -globulin content of the plasma. In some instances, considerable plasma protein (up to 20 gm. daily) is diverted to the ascitic fluid, but production of plasma protein by the diseased liver is also abnormal. In surgical shock, the hemoglobin content of blood increases and the plasma proteins fall, owing to increased transfer of plasma protein and water to the tissue fluids. Plasma fibrinogen is usually low during acute hepatic necrosis (acute yellow atrophy, severe arsphenamine hepatitis, and poisoning by carbon tetrachloride, chloroform, or phosphorus), also in cachectic conditions and typhoid fever, and following severe hemorrhage. The hypoprothrombinemia of hepatic disease is discussed on pages 67 and 673. Adequate protein and carbohydrate intake is important for optimal liver repair and regeneration. Hypoproteinemia and depletion of hepatic protein increase the toxic effects of chloroform, and favor the development of extensive hyaline necrosis. Malnutrition resulting from gastro-intestinal disease, malignancy, tuberculosis, improperly treated diabetes mellitus, or prolonged

low protein intake produces hypoproteinemia, hypoalbuminemia, and an accompanying nutritional edema. The dehydration which accompanies severe diabetic acidosis can result in sufficient hemoconcentration to mask the plasma protein deficit. Such diabetic patients become edematous after active treatment; administration of fluid and saline restores the plasma volume and allows the development of hypoproteinemia. Hypoproteinemia may be expected in the toxemias of pregnancy (pernicious vomiting, eclampsia) and during lactation, if synthesis of plasma protein is inadequate to balance that withdrawn by the mammary glands. During normal pregnancy, the plasma protein concentration decreases to 6 per cent. This reduction, which involves chiefly the albumin fraction, is attributed to an "internal plasmapheresis" or transfer of plasma proteins to the fetus. Moderate hypoalbuminemia occurs in both hypothyroidism and hyperthyroidism, with compensatory increase in one of the globulin fractions.

Marked decrease in plasma albumin is frequently accompanied by an increased globulin concentration, in which case the albumin : globulin ratio is decreased, or reversed. The intimate relation of hypoalbuminemia to edema is due to the oncotic pressure of plasma albumin. Edema may be expected whenever serum albumin is less than 2.5 per cent, plasma protein below 5.0 per cent, or plasma specific gravity less than 1.023. The critical plasma albumin level in nephrotic children is approximately 1.2 per cent. Transfusion, or intravenous injection of plasma or serum albumin, temporarily relieves such edema; nephrotic patients excrete a considerable fraction of injected serum albumin in their urine. Intravenous administration of gum acacia is less desirable; it increases the oncotic pressure temporarily but overdosage can cause hepatic damage, inhibit the regeneration of plasma protein, and aggravate the hypoproteinemia. The presence of gum acacia in the liver has been detected at autopsy six years after administration of the substance. Repeated intravenous injections of gelatin may also result in hypoproteinemia and intoxication. In nephrosis and malnutrition, the edema fluid tends to have a low protein content, but in acute glomerulonephritis the capillary permeability is generally increased and the edema fluid contains more plasma protein. Hypoproteinemia retards wound healing, and extreme depletion of plasma protein causes hypothermia, increased catabolism of tissue proteins, and collapse (shock). Hepatectomy reveals a considerable increase in the protein catabolism of the peripheral tissues in experimental hemorrhagic shock. Burned patients can exhibit a daily excretion of 45 gm. of urinary nitrogen, a large fraction of which is polypeptide nitrogen. Injection of plasma or serum albumin is very beneficial in shock, burns, and non-healing surgical wounds; saline or glucose should be injected also if dehydration is present. Methionine feeding reduces the urinary loss of nitrogen in burned rats.

HYPERPROTEINEMIA

Severe, rapid dehydration can increase the plasma albumin concentration to 10 or 12 per cent, and can elevate the plasma globulin level. Hemoconcentration accompanies diabetic acidosis, extensive burns, severe diarrhea, vomiting, gastro-intestinal obstruction, and marked restriction of fluid intake. The protein content of plasma and tissue fluids is increased in myxedema. Plasma α -globulin rises in fevers, hypothyroidism, severe diabetes mellitus, and nephrosis, and β -globulin is elevated in cirrhosis, hyperthyroidism, and nephrosis. γ -Globulin can increase independently of the other plasma proteins, in such acute infectious or suppurative processes as lung abscesses, bacterial endocarditis, osteomyelitis, pneumonia, rheumatic fever, and aplastic anemia, also in primary hepatocellular disease, kala-azar, leprosy, lymphogranuloma inguinale, granuloma inguinale, disseminated lupus erythematosus, Boeck's sarcoid, schistosomiasis, Still's disease, and multiple myeloma. In the latter condition, Bence-Jones protein, whose electrophoretic behavior resembles that of β -globulin, appears in the plasma, and the protein level may be as high as 18 per cent. The serum of patients with kala-azar or endocarditis lenta contains a globulin which flocculates at low temperatures. Plasma fibrinogen rises during acute infections (other than typhoid fever), inflammatory conditions, trauma, hepatitis, lymphogranuloma inguinale, granuloma inguinale, nephrosis, multiple myeloma, Boeck's sarcoid, menstruation, pregnancy, and after irradiation with x-rays. In malaria, the serum euglobulin fraction increases, and it shows increased reactivity in the melanoflocculation test and with the Folin and Ciocalteu phenol reagent. While these tests are non-specific, they are more sensitive than microscopic examination of blood films for the laboratory diagnosis of malaria.

A number of qualitative tests have been devised to detect increase or qualitative change in plasma globulins. Most widely used for detection and prognosis of acute hepatocellular damage is the *cephalin-cholesterol flocculation test*. Flocculation of the cephalin-cholesterol emulsion is attributed to increase or alteration in serum γ -globulin; positive reactions are therefore obtained also in some of the extrahepatic diseases which elevate the γ -globulin fraction of plasma. (See above.)

ALBUMINURIA (PROTEINURIA)

Normal human urine contains only from 1 to 6 mg. per cent protein — chiefly albumin and sulfomucin. Abnormal concentrations can be detected by heating the urine with a mixture of sodium chloride and acetic acid, or by the flocculating action of sulfosalicylic acid or concentrated nitric acid. In *Heller's test*, the urine is stratified carefully over concentrated nitric acid; a ring of white flocculate indicates the presence of

albumin. Concentrated urines may give an opaque zone of crystalline uric acid or urea nitrate, which can be avoided by proper dilution of the urine. Resin acids of certain drugs give similar artifacts that can be differentiated by their solubility in alcohol. The production of a deeply colored zone may be avoided by using Robert's reagent (1 volume concentrated nitric acid and 5 volumes saturated magnesium sulfate). Urinary albumin can also be detected by heat coagulation after adding one-sixth volume of 33 per cent acetic acid to the urine. Bence-Jones protein gives the albumin flocculation reactions, but it redissolves near the boiling point. Urinary albumin is reported clinically as follows: A very faint precipitate as +; a moderate precipitate, equivalent to 0.1 per cent albumin, as ++; a heavy opaque precipitate, equivalent to 0.25 per cent albumin, as +++; and a very dense precipitate, equivalent to 0.5 per cent albumin or more, as ++++.

The term *albuminuria* is commonly used to signify abnormal proteinuria. Globulin and, at times, fibrinogen are excreted simultaneously with the albumin. The albumin constitutes the major fraction of the excreted protein. In lipid nephrosis, nephrosclerosis, and functional albuminuria, the albumin : globulin ratio of the urinary protein may exceed 10; in glomerulonephritis it is usually between 3 and 8, and in amyloid disease between 0.5 and 3.5. Lowering of the albumin : globulin ratio indicates increased permeability of the renal barrier. Intravenous injection of foreign proteins leads not only to the partial elimination of these proteins by the kidney, but also to true albuminuria. The plasma proteins of nephritic and nephrotic patients exhibit modified serological responses to antisera for normal plasma proteins. The molecular weights of these proteins are reported to be higher than normal, while the molecular weights of the urinary proteins are comparatively low. A portion of the serum albumin in patients with amyloidosis and nephrosis has a sub-normal cystine content. The glomeruli are primarily responsible for the excretion of both plasma proteins and foreign proteins, despite the anatomical prominence of lesions in the renal tubular epithelia of nephrotic kidneys.

Functional or benign albuminuria is a slight transitory proteinuria, most commonly found in young persons. As a rule, less than 1 per cent of protein is found in the urine, and the condition has little pathological significance. Functional albuminuria may be provoked by severe exercise, mental strain, prolonged exposure to cold, or the ingestion of egg white. The most frequent type, known as *essential albuminuria* (also cyclic, orthostatic, or postural albuminuria), is caused by a disturbance of the renal circulation when the patient assumes an upright position. It may be due to hereditary abnormalities, lordosis, movable kidney, or to vasomotor instability. The condition can be confused with more serious renal disease, if urine albumin tests are not supplemented by other examinations. Temporary albuminuria from renal congestion occurs during pregnancy and

labor and prior to menstruation; albuminuria is more marked in the toxemias of pregnancy.

Organic albuminuria may result from passive congestion of the kidneys, due to cardiac decompensation, ascites, or intra-abdominal tumors. This type of albuminuria usually disappears when the circulatory efficiency is improved. Convulsive disorders and coma associated with cerebral vascular damage incite albuminuria. Other conditions which cause albuminuria by secondary kidney damage or interference with renal nutrition include acute intestinal obstruction, severe anemias, diabetic coma, hyperthyroidism, obstructive jaundice, leukemia, purpura haemorrhagica, and fevers (especially childhood infections, malaria, pneumonia, tuberculosis, and rheumatic and typhoid fevers). In jaundice, an accumulation of bile salts and pigments alters the embatic behavior of plasma proteins, and causes irritation of kidney tissue. Arsenic, cantharides, copaiba, mercury, phenol, quinine, salicylic acid, and turpentine produce renal irritation and albuminuria.

The renal lesions resulting from carcinoma, hypernephroma, glomerulonephritis, nephrosis, tuberculosis, and infarction cause *renal albuminuria*. The albuminuria which accompanies acute glomerulonephritis tends to continue during the chronic stage of the disease, until glomerular impairment becomes so extensive as to hinder the excretion of proteins. Marked continuous albuminuria is an important symptom of nephrosis, and it may aggravate tubular damage. In necrotizing nephroses, caused by poisonous metals or hypnotics, anuria may intervene and stop the albumin excretion. In lipid nephrosis and amyloid disease, the urine usually contains from 2 to 5 per cent protein; superimposed glomerulonephritis can diminish the proteinuria subsequently. The average daily excretion of plasma protein during chronic nephrosis is from 15 to 20 gm., although losses as high as 110 gm. have been reported. The albuminuria is increased by a high protein diet. In glomerulonephritis, nephrosclerosis, and essential hypertension, the urine usually contains less than 1 per cent protein, and the albuminuria is inconstant. The degree of albuminuria does not always parallel the kidney damage.

Postrenal or false albuminuria results from degenerative or inflammatory lesions of the renal pelvis and lower urinary passages, and from admixture of vaginal secretions or discharges. Urine which is mixed with blood or pus will contain albumin from these sources; approximately 0.1 per cent of albumin may be expected for each 50,000 leukocytes per ml. of urine.

OTHER PROTEINURIAS

Sulfomucin, which flocculates like nucleoprotein, is excreted in abnormal quantity in the urine during nephritis and inflammation of the urinary passages (cystitis and pyelitis). It forms the hyaline casts and is present in other casts. Substances which flocculate like proteoses are excreted

during carcinoma, fevers, and osteomalacia. One of these, the *Bence-Jones protein* (liberated from bone marrow, lymphocytes, and leukocytes), is produced in excessive quantities and excreted with plasma proteins in some cases of multiple myeloma, myelogenic osteosarcoma, Hodgkin's disease, lymphosarcoma, empyema, and leukemia. Continued excessive excretion of Bence-Jones protein causes kidney damage. Flocculated Bence-Jones protein dissolves at 100° C., and reflocculates on cooling. The molecular weight of this protein is approximately one half that of serum albumin or of hemoglobin. When hemoglobin appears in the plasma in sufficient concentration, it is readily excreted by the kidney. Hemoglobinuria is considered on page 560.

AZOTEMIA

This term is usually applied to an increased NPN (non-protein nitrogen) of blood; it may be extended conveniently to include increased blood urea concentration, inasmuch as urea nitrogen constitutes the major fraction of the NPN. Increases in urea nitrogen and NPN usually have the same clinical significance. Both rise in nephritis and other azotemic conditions (except eclampsia, gout, myelogenous leukemia, and multiple myeloma, where increases in the NPN are due partly to increased uric acid levels). Repeated blood NPN determinations are important to ascertain whether or not the azotemic condition is improving.

The azotemia of renal disease is due to impaired excretory ability, prerenal diversion of water from the blood stream, and excessive protein catabolism caused by infections, dehydration, and so forth. As little as one fourth of the kidney tissue can maintain normal blood nitrogen levels, provided prerenal factors are favorable. As long as diseased kidneys can compensate their deficient concentrating power by secreting a large volume of dilute urine, the NPN does not rise. Dehydration is an important extrarenal azotemic factor in acute nephritis, chronic hypertensive nephritis, and necrotizing nephroses. Diarrhea, hemorrhage, prolonged vomiting, excessive perspiration, burns, surgical shock, diabetic coma, and the administration of purgatives induce dehydration and azotemia. Dehydration, azotemia, and marked oliguria can result from acute intestinal obstruction, adynamic ileus, Addison's disease, hyperparathyroidism, myocardial failure, reflex or postoperative urinary suppression, and prostatic or ureteral obstruction. The NPN can rise during rapid removal of edema fluid, inasmuch as water is more readily excreted than the nitrogenous substances. Retention of nitrogen is, at times, precipitated by low protein intake; high protein diets increase the blood nitrogen levels of nephritics only temporarily. Usually, azotemia accompanied by the secretion of urine of low specific gravity is of serious import (page 450).

Azotemia has slight prognostic value in acute nephritis, even though the blood urea nitrogen may rise to 300 mg. per cent and the NPN to 400 mg.

per cent. In early nephritis, azotemia tends to be periodic and depends largely on extrarenal influences, while in advanced chronic nephritis, azotemia becomes progressive. In late stages, the urea nitrogen and the NPN remain permanently above 80 and 100 mg. per cent, respectively. Permanent azotemia is less common in nephrosclerosis, which usually terminates in cardiac or vascular failure. The NPN is often normal in chronic nephrosis, and the presence of azotemia suggests a complicating nephritis, increased endogenous catabolism, or dehydration from rapid dissipation of edema fluid. Azotemia can result from destruction of renal tissue, as in congenital polycystic kidneys, hydronephrosis, malignancy, congenital renal hypoplasia, and renal tuberculosis.

A moderately increased NPN and blood urea, of extrarenal origin, result from gastro-intestinal hemorrhage (due largely to digestion, transport, and accelerated urea formation), from excessive endogenous catabolism of severe febrile or toxic conditions, and, occasionally, as a sequel to biliary surgery or terminal hepatic disease. Blood urea nitrogen above 50 mg. per cent indicates a serious prognosis in hematemesis or melena; re-establishment of a normal level within a few days following gastro-intestinal hemorrhage is a favorable sign. Blood nitrogen determinations are of considerable value to the surgeon in deciding the advisability and optimal occasion for surgical intervention; marked azotemia indicates a poor surgical risk. When urinary obstruction is not complicated by chronic renal disease, relief of the obstruction and institution of adequate drainage frequently return the blood nitrogen to a normal level.

HYPOAZOTEMIA

Blood urea nitrogen may be as low as from 5 to 10 mg. per cent in severe hepatic insufficiency (acute yellow atrophy, hepatic necrosis caused by poisons, or eclampsia). During the first six months of pregnancy, the NPN tends to decrease about 5 mg. per cent; near term the level returns to normal.

RETENTION OF OTHER NITROGENOUS SUBSTANCES

The *creatinine* content of blood increases temporarily during acute nephritis, prostatic obstruction, and bilateral ureteral obstruction. In chronic glomerulonephritis the blood creatinine tends to be normal until an advanced, terminal, or uremic stage is reached, when values as high as 35 mg. per cent may be attained. In chronic nephritis, creatinine levels above 5 mg. per cent indicate a grave prognosis; increased creatinine levels do not have this significance in acute nephritis. Blood *creatinine* increases occasionally in severe nephritis, and in patients exhibiting creatinuria (page 462).

The *amino acid nitrogen level* of the blood is maintained with remarkable constancy. It is increased in some cases of eclampsia, myelogenous

leukemia, advanced diabetes mellitus, late uremia, and severe hepatic necrosis from acute yellow atrophy, yellow fever, or poisoning by arsphenamine, carbon tetrachloride, chloroform, cinchophen, or phosphorus. In these conditions, the blood amino acid nitrogen may rise to 10 or 15 mg. per cent. One case of acute yellow atrophy has been reported in which the premortal blood amino acid nitrogen was 200 mg. per cent, owing to deficient hepatic deamination. In most hepatic and renal diseases, the blood amino acid level is normal; the liver has a great functional reserve and marked regenerative powers. The blood level of amino acids is lowered in nephrosis of childhood and in the acute stage of pneumonia. The *phenols* of blood and tissue fluids are increased in uremia. Certain uremic symptoms have been attributed to retained phenol (page 456); indican accumulation is regarded as an indication of approaching uremia. Deproteinized nephritic serum reacts abnormally with the Van den Bergh bilirubin reagent; an orange color is produced, which turns to an evanescent pink in the presence of alkali. This diazo test for nephritis indicates an indican (or indoxylglycuronate) level above 1.5 mg. per cent. The test is usually positive only in advanced renal insufficiency; it cannot be performed in the presence of hyperbilirubinemia. The *guanidine* of blood is increased in uremia. The *residual or undetermined nitrogen* sometimes increases independently of urea during normal pregnancy and eclampsia; it also rises disproportionately to the NPN in some cases of uremia or burns. The nitrogenous compounds responsible for these effects have not been determined.

CONCENTRATION TESTS OF RENAL EFFICIENCY

Retention of water, salts, and nitrogenous metabolites is dependent on renal function and extrarenal factors. (For details of water and salt excretion, see Chapter VIII.) The tubular epithelium of a diseased kidney has a limited reabsorbing or concentrating ability. Determination of the latter is of greater assistance than balance studies in nephritic conditions, because the diseased kidney can establish a polyuria, which compensates its deficient concentrating power. Inability to perform normal concentration is termed *hyposthenuria*. It can be produced in dogs by a low protein diet, uranium poisoning, renal denervation, ureteral obstruction, or reduction of kidney mass. As the nephritic condition progresses, the specific gravity of the urine tends to become fixed near that of protein-free blood plasma (1.007). When the urinary maximal specific gravity is 1.010 or less, selective reabsorption in the renal tubules has practically ceased.

In the *Mosenthal concentration test* variations in the urinary specific gravity and volume are determined for a period of 24 hours. The patient is given three meals, which contain a total of approximately 85 gm. protein, 8.5 gm. sodium chloride, and 1,750 ml. fluid; no food or fluid is permitted between meals. Six two hour urine specimens are collected during the

course of the test day and one twelve hour sample the following morning. The corrected specific gravity of each sample is determined, 0.003 being subtracted for each 1 per cent of albumin in the urine. The maximal variation between the several specific gravity readings is 0.010 or more, and the total volume of the day samples is from three to four times that of the night sample in normal persons. As renal disease develops, the night volume increases and the urinary specific gravity becomes fixed at a relatively low level. In late stages, compensatory polyuria fails, and retention and azotemia develop. The concentrating ability is not always impaired during acute nephritis; azotemia under these circumstances is the result of accelerated protein catabolism. Diabetes insipidus and polyurias caused by inflammation of the urinary tract must be differentiated from renal impairment. Low fixation of urinary specific gravity may also result from marked discharge of edema fluid, or the forced administration of fluids. A high fixed specific gravity indicates prerenal diversion of water (as in myocardial failure), with a normal ability to concentrate urine.

The *Fishberg concentration test* is performed as follows: at 6 P.M., a high protein meal, which includes less than 200 ml. of fluid, is given, but no other fluid or food is permitted until the test is completed. Urine is discarded until 7 A.M.; then three successive one hour samples are collected and their specific gravities determined. Normally, at least one of these samples will have a specific gravity greater than 1.022; but as renal function is progressively impaired, the maximal specific gravity approaches ever closer to 1.007.

In the *urea concentration test*, 15 gm. urea and from 100 to 150 ml. water are given orally, and urea concentrations are determined in two successive one hour urine specimens. If a urea concentration of 2 per cent is not found in one of the samples, renal impairment is indicated. This test does not provide a quantitative estimate of the extent of renal damage; it may even give false indications in edematous patients, because of the diuretic action of the administered urea.

A concentration test and determination of the NPN give most of the qualitative diagnostic information desired by the physician. The concentration tests detect functional renal impairment, while the NPN values show whether a renal condition is compensated or uncompensated. Quantitative information concerning the degree of kidney impairment is given by the urea clearance test (page 452). This test is perhaps most useful for detecting changes in the renal functional condition of a patient. When Mosenthal's concentration test reveals urinary specific gravity above 1.026, there is no need for the urea clearance test. Both the Mosenthal test and the urea clearance test detect earlier stages of renal impairment than does blood analysis or the phenolsulfonphthalein test. During convalescence from acute nephritis, concentration tests reveal residual renal impairment after the urea clearance has returned to normal.

CLEARANCE TESTS OF RENAL EFFICIENCY

The widely used *phenolsulfonphthalein* (*phenol red*) test is conducted as follows: The bladder is emptied, from 200 to 250 ml. of water are given orally, and fifteen minutes later 6 mg. of the dye are injected intravenously or intramuscularly. Seventy minutes later, the first urine specimen is collected, and, following this, a second one hour specimen. Ten per cent sodium hydroxide is added to each urine sample until maximal colors develop, and then each sample is diluted to 1,000 ml. The dye content is determined by colorimetric comparison against a 0.3 mg. per cent solution of phenol red. Normally 50 ± 10 per cent of the injected dye is excreted in the first urine sample and 22.5 ± 2.5 per cent in the second sample. When less than one half the dye appears in the combined samples, renal impairment is indicated. However, prerenal diversion of water can cause false low values, and the compensated and uncompensated forms of renal disease are not differentiated by the test. In the presence of hepatic disease, false values are produced by diversion to the urine of that fraction of the dye which is normally excreted in the bile. A particular advantage of the test in the investigation of surgical conditions of the kidneys is the ease of separate determinations of the functional capacities of the two kidneys by the use of ureteral catheters or by direct observation through a cystoscope. A diseased kidney will not begin to secrete the dye within the normal time (from three to five minutes after intravenous injection, or from five to ten minutes after intramuscular injection), and the diseased organ eliminates the dye more slowly than the healthy kidney. Retention of phenol red usually parallels the degree of azotemia. In the blood stream, 85 per cent of the phenol red is combined with plasma proteins. The dye is excreted principally by the renal tubules, with an average clearance of 400 ml. per minute in man. Diodrast, neutral red, and indigo carmine are also excreted by the tubules.

The *urea clearance test* provides information not obtainable from separate blood and urine examinations, namely, an estimate of the degree of renal impairment in compensated cases of nephritis. Since the normal individual variation in urea clearance is greater than that of carefully managed concentration tests, it is most useful in detecting a change in renal function. The test is conducted on resting patients as follows: Two or three glasses of water are taken after a night's fast. The bladder is then emptied, the urine discarded, and breakfast is given (or the test may be started at 10 A.M. by taking one glass of water at the beginning of the test and another one hour later). Exactly one hour after emptying the bladder, the first urine collection is made and a blood sample is taken; at the end of the second hour, another urine specimen is collected. The urine volumes are measured, and the blood and urines are analyzed for urea (plus ammonia). The rate of urea excretion varies directly as the square root of the blood urea concentration, and inversely as the square root of the urinary urea

concentration. Urea clearance is expressed as the milliliters of blood completely cleared of urea by the kidney. (The urea is actually removed from a greater volume of circulating blood; only one tenth of the blood urea is excreted during a single passage of the blood through the kidney.) When urine is formed in excess of 2 ml. per minute, the clearance is *maximal* and it averages 75 ml. (from 60 to 100 ml.) of blood per minute. When less than 2 ml. of urine are secreted per minute, a *standard clearance* approximating 54 ml. (from 40 to 65 ml.) per minute results. These urea clearances are formulated as follows (the average value of the two urine samples being used):

$$\text{Maximum clearance} = \frac{\text{mg. per cent urea in urine} \times \text{ml. urine per minute}}{\text{mg. per cent urea in blood}}$$

$$\text{Standard clearance} = \frac{\text{mg. per cent urea in urine}}{\text{mg. per cent urea in blood}} \times \sqrt{\text{ml. urine per minute}}$$

Results are usually expressed as percentages of the normal clearance values given above. The calculations are valid for persons between 62 and 71 inches in height. Urea clearance is low in the newborn; for children, the urinary volume is multiplied by

$$\frac{1.73}{\text{sq. m. of body area}}$$

and this corrected value is used in the calculations. If the urine secretion is less than 20 ml. per hour, the urea clearance test gives erroneous values.

The maximum urea clearance is increased by acute infections, meals, caffeine, and small doses of adrenaline. It is decreased by exercise, low protein intake, avitaminosis A, pituitrin, large doses of adrenaline, and the low blood pressure of cardiac failure or shock. Whenever the urinary flow is obstructed, the clearance is low, owing to increased reabsorption of urea by the tubules. In nephritis, the urea clearance parallels the number of functioning glomeruli; it is not diminished by tubular damage alone. Urea clearance decreases in most cases of acute nephritis, and it begins to be restored within four months in those cases which recover. The clearance remains consistently lower than 60 per cent in chronic nephritis; by the time retention appears and the NPN rises, the urea clearance has decreased to 50 per cent or less. In terminal stages it is usually below 20 per cent, and in uremia it is 5 per cent or less. Administration of salyrgan or xanthine diuretics usually does not alter urea clearance in nephritic patients. Urea clearance may remain normal for long periods during nephrosis, essential hypertension, and arteriosclerotic disease; but renal insufficiency can also develop suddenly in malignant hypertension. The clearance is usually normal in eclampsia.

The *creatinine clearance* gives a more accurate estimate of glomerular filtration than does urea clearance, although when the creatinine blood

level is low, a small quantity of creatinine is excreted by the tubules. In the creatinine clearance test, from 3 to 5 gm. of creatinine are given orally in 400 ml. of water. Blood samples are taken at one and two hours. Urine is collected during the one hour interval between the blood samples. The clearance is calculated as follows:

$$\text{Creatinine clearance} = \frac{\text{urine creatinine conc.}}{\text{av. blood creatinine conc.}} \times \text{ml. urine per minute}$$

The normal clearance is 150 ± 50 ml. per minute; values below 60 are considered pathological. Thyroxine increases the creatinine clearance.

Ferrocyanide, inulin, sucrose, xylose, inorganic sulfate, and diodrast have been used in similar clearance tests. Ferrocyanide behaves like urea and uric acid in man; about 40 per cent of these substances are passively reabsorbed by the tubules. The clearances of diodrast, hippuran, phenol red, sulfate, acetylsulfonamides, inulin, sorbitol, mannitol, dulcitol, sucrose, glucose, and xylose are higher than the urea clearance. Sucrose and xylose clearances are approximately 100 ml. per minute; the clearances of inulin, creatinine, sulfate, acetylsulfonamides, sorbitol, mannitol, and dulcitol are even higher, since their tubular reabsorption is minimal. The maximal rate of tubular reabsorption (T_m) of glucose, which is approximately 345 mg. per minute in the normal adult, is a measure of the reabsorptive capacity of the tubules. When the reabsorption of sugars is inhibited by phlorhizin, the clearances for glucose, xylose, and sucrose approach that of inulin. The diodrast and inulin clearance tests are sensitive measures of early renal changes. *Inulin clearance* is probably the truest measure of glomerular filtration; it approximates 125 ml. per minute in a normal adult. The inulin clearance is increased by injection of pyrogens, and it is decreased by hypovitaminosis A and by hypophysectomy. It is about 30 per cent below normal during the first week of life, owing to immaturity of the renal glomeruli in infants. Inulin clearance is subnormal in early stages of glomerulonephritis, and it decreases as the disease progresses. Acetylsulfanilamide has a clearance equal to that of inulin, while the clearance of sulfanilamide is only 55 ml. per minute. When sufficient inorganic sulfate is injected to give a blood level of 190 mg. per cent, the sulfate clearance is approximately 110 ml. per minute. *Diodrast clearance* is a measure of the renal plasma flow; it is about 700 ml. per minute in normal men, and it decreases in glomerulonephritis. Since diodrast is excreted rapidly by the tubules, its maximal tubular excretion (T_{mD}) is an index to the number of active tubules (page 573). In man, it is about 52 mg. of diodrast iodine per minute. The clearances of hippuran, and of the *p*-amino and *p*-hydroxy derivatives of hippuric acid, simulate the diodrast clearance, and like the latter are decreased in glomerulonephritis.

NEPHRITIS

Nephritis, or Bright's disease, is a progressive inflammatory, proliferative and degenerative lesion of the kidney. There are three prominent nephritic syndromes, namely, interstitial glomerulonephritis, nephrosis (or parenchymatous nephritis), and nephrosclerosis (or arteriosclerotic nephritis). The first syndrome is characterized by hyposthenuria, nitrogen retention, a tendency toward uremia, variable albuminuria, edema, and hypertension. In nephrosclerosis, the predominant characteristics are arterial hypertension and cardiovascular disturbances, while in the degenerative nephroses, the cardinal symptoms are edema and albuminuria.

The early lesions of acute glomerulonephritis include swelling and proliferation of the glomerular endothelial cells with accumulation of inflammatory exudate in the loops. This tissue reaction is related to immunization and hypersensitivity, and it can be produced by injection of antikidney serum. Clinical nephritis is frequently initiated by toxins of bacteria (particularly streptococci), which affect the kidney diffusely. It may follow upper respiratory tract infections, contagious childhood disease, pneumonia, syphilis, tuberculosis, or typhoid fever, and it is present in some cases of toxemia of pregnancy. Acute glomerulonephritis in children often leaves very little residual renal damage. Nephritis can be produced experimentally by the administration of arsenic, mercury, and uranium salts, chromates, tartrates, cantharidin, etc. The protein intake has little relation to the onset of kidney pathology. High protein diets merely produce renal hypertrophy of a physiological nature. Eskimos, who daily ingest 500 gm. or more of protein, show no predilection for renal diseases. The acute stage of glomerulonephritis is attended by profuse albuminuria, hematuria, marked deficiency of renal function, and edema (partly due to increased capillary permeability). In the chronic stage, erythrocytes and leukocytes are frequently found in the urine.

Uremia is a syndrome associated with retention of urinary metabolites in advanced glomerulonephritis. Uremic conditions can be induced by nephrectomy, deficiency of desoxycorticosterone, and excess of parathormone. The symptoms include urinous odor of the breath, cachexia, anemia, hyposthenuria, gray color, skin eruptions, vomiting, gastritis, enteritis, hypertension, retinitis, heart failure, dyspnea, Cheyne-Stokes respiration, muscle twitching, headache, hiccoughs, psychoses, apathy, and either deep coma or convulsions at death. Dogs with ligated kidneys become listless by the second day; vomiting, tremor, and muscle twitching appear during the third day, and death occurs by the fifth day. An otherwise normal human being, whose single accidentally ruptured kidney was removed, lived ten days. There was no change in temperature or cardiac involvement, other than a fall in diastolic pressure. Drowsiness, headache, and muscle twitching appeared on the fifth day when the NPN was 200

mg. per cent. Coma, vomiting, and acidosis were well developed by the sixth day. During the last day of life, the blood values were: NPN, 268 mg. per cent; sodium chloride, 351 mg. per cent; calcium, 7.9 mg. per cent; inorganic phosphorus, 14.8 mg. per cent; and hemoglobin 7 per cent. The total plasma protein was near normal, but the albumin : globulin ratio was only 0.77. Convulsions, edema, hyperpnea, stomatitis, or diarrhea did not develop. These uremic symptoms are apparently complications of extrarenal origin. When edema occurs in the uremic stage of nephritis, it is usually due to congestive heart failure rather than to hypoproteinemia.

Uremic symptoms are attributable to disturbed metabolism of more than one substance. The acidosis is caused partly by retention of phosphate, sulfate and organic acids, failure of the ammonia mechanism, loss of base, and dehydration. The low blood chloride is the result of diarrhea and vomiting. Increasing blood phenol and serum phosphate levels are better indications of approaching uremia than is the NPN. The increased inorganic phosphate and magnesium of plasma cause a lowering of the serum calcium ion concentration. The latter, and the increase in blood guanidine, cause motor irritation. Accumulation of unconjugated phenol indicates deranged liver function, and it may be partly responsible for the apathy and cardiac failure of uremia. The urinous odor of the breath is caused by bacterial formation of ammonia from urea in the saliva. The gastro-intestinal symptoms may also be related to production of ammonia by urease of the gastric juice, or, at times, to cerebral edema.

In uremia, the urea clearance is usually less than 5 per cent of normal, and less than 10 per cent of the injected test dose of phenolsulfonphthalein is eliminated within 2 hours. Creatinine, urea nitrogen, and NPN are in excess of 5, 70, and 100 mg. per cent, respectively; serum inorganic phosphorus is above 7 mg. per cent, and serum calcium is usually slightly low. The blood cholesterol may be quite low, as the result of cachexia and anemia; the fasting blood sugar may be increased.

NEPHROSIS

The nephroses include the characteristic primary lipid nephrosis; necrotizing nephroses resulting from poisoning by arsenical drugs, bismuth, mercury, or hypnotics; amyloid nephrosis (amyloidosis); and the larval nephroses caused by fevers, chronic suppuration, pernicious anemia, diabetes, hyperthyroidism, jaundice, the toxemia of pregnancy, and drugs. Intravenous administration of excessive quantities of sucrose can produce tubular degeneration and nephrosis. The nephroses are frequently characterized by degenerative tubular lesions and renal cholesterol ester deposits. At times, nephrosis represents an early stage of chronic nephritis. Prominent symptoms of nephrosis include extensive edema, albuminuria, hypoproteinemia, hypoalbuminemia, lipemia, cholesterolemia, oliguria, hypo-

calcemia, lipuria, and, frequently, a low basal metabolic rate. Such nephritic signs as azotemia, hematuria, hypertension, renal insufficiency, and lesions of the glomeruli are absent in early stages of nephrosis, but they may develop if chronic glomerulonephritis ensues. While nephrosis may continue for years without functional impairment of the kidney, there is a tendency to develop nephritic lesions. Loss of plasma albumin in the urine, and a variable interference with the regeneration of plasma protein, account for the severe edema. The striking increase in blood cholesterol and cholesterol esters is associated with the hypoproteinemia.

Amyloidosis or amyloid nephrosis results from long-continued cachexia and suppuration, as in chronic tuberculosis, and, at times, in Hodgkin's disease, empyema, lung abscess, osteomyelitis, pyelonephritis, and syphilis. Marked deposition of the abnormal iodine-staining protein, amyloid, occurs in the spleen, kidney, and liver. Renal amyloidosis is accompanied by albuminuria, casts, hyposthenuria, hypoproteinemia, and edema. The *Congo red test* for amyloidosis is based on the rate of clearance of the intravenously injected dye, as estimated by colorimetric examination of the centrifuged blood. Normally, only from 10 to 30 per cent of an injected dose of 100 mg. of Congo red is removed from the blood plasma within one hour, whereas approximately 60 per cent is removed in amyloid patients. A 20 mg. dose of the dye disappears from the blood of an amyloidosis patient within 15 minutes. Congo red is excreted principally in the bile; its rapid removal from the blood of amyloid patients is credited to hypoproteinemia and damage to capillary endothelia. Similar rapid removal of Congo red occurs in lipid nephrosis, but in this case a considerable fraction is transferred to the urine, owing to the embatic effect of the excreted serum albumin. The diagnosis of amyloid disease is, therefore, based on a rapid lowering of the blood Congo red level without significant elimination in the urine. Because of variable albuminuria, consistent results are not always obtainable, and the test may be negative in the presence of slight amyloidosis.

Treatment of Nephritis and Nephrosis

High carbohydrate diets should be administered to nephritics to spare their tissue protein, particularly when uremia is impending. Fat is tolerated poorly, owing to gastro-intestinal disturbances. Base-forming foods are important to combat the acidosis; acid ash diets may aggravate the kidney pathology. The diet should contain sufficient protein of high biological value to provide at least 1 gm. per kg. of body weight, in order to counteract excessive tissue wasting and anemia. In acute nephritis, and during the progressive nitrogen retention of chronic nephritis, the protein intake is curtailed to about 25 gm. daily. At times, water restriction and low salt diets are used to treat the edema. The diet ordinarily employed for this purpose is one in which sodium chloride is used sparingly

in the preparation of the food and none is provided at the table. This limits the salt intake to from 2 to 4 gm. daily. Rest is very important in treating nephritis.

The metabolic treatment of uremia includes parenteral administration of saline and glucose, and later a high carbohydrate, low protein diet, to counteract dehydration, promote urine flow, and relieve malnutrition and hepatic hypofunction. Calcium and magnesium salts can be injected to decrease muscular irritability. Sweating, laxatives, and diuretics are disappointing measures for the compensation of renal insufficiency. Even though the nitrogen excretion through the skin and intestine can be increased by these procedures, the accompanying excessive elimination of water tends to cause dehydration and elevation of the NPN. Diuretics are often ineffective; digitalis may be beneficial when cardiac complications are present.

Nephrosis often responds favorably to a high caloric, high protein diet. Nephrotic patients can store large quantities of protein for long periods in the tissue fluids and liver. As much as 150 gm. protein may be given to adults, daily, to combat the protein wastage in this disease. Rest, restriction of salt and water, and the administration of ammonium chloride are other measures employed to reduce nephrotic edema.

NEPHROSCLEROSIS, HYPERTENSION, AND ECLAMPSIA

In nephrosclerosis, the cardinal symptoms are cardiac hypertrophy and high blood pressure, whereas the renal function may be impaired only moderately for long periods. Hypertension also occurs independent of ordinary renal symptoms; this condition, known as essential hypertension, can become malignant, and may cause death by cardiac failure or cerebral hemorrhage. Such forms of hypertension are now considered to be related to renal hypertension, which is accompanied by progressive destruction of kidney parenchyma. According to inulin clearance determinations, the glomerular filtration rate remains normal in most hypertensive patients. The diodrast clearance, and hence the renal plasma flow, tend to decrease because of humoral vasoconstriction. The maximal tubular diodrast excretion is lowered early in hypertension, and it decreases as ischemia and tubular impairment of the kidney become more pronounced. Renal hypertension is not produced by bilateral nephrectomy but does result from kidney damage or renal ischemia. The renal blood flow must be reduced by 40 per cent to induce experimental hypertension in dogs. Removal of the affected kidney, in unilateral renal disease, allows the blood pressure to return to normal in approximately 6 hours. Hydronephrotic and ischemic kidneys, and those of hypertensive and adrenalectomized dogs, contain an excess of an enzymatic hormone (a cystine-free globulin) termed *renin*, which is liberated into the blood stream. This enzymatic protein is probably produced by myoepitheloid cells in the glomerular

segments of the afferent arterioles (although some believe it is made in the tubules). It is absent from aglomerular kidneys. The myoid cells undergo hypertrophy and hyperplasia as the result of renal ischemia. Renin acts on a heat-labile α -globulin (pseudoglobulin) "activator" or substrate of blood plasma and lymph to form the active pressor substance, *angiotonin* or *hypertensin*. The renin activator, protein substrate, or *hypertensinogen* is formed in the liver. Hypertensin has been isolated as a crystalline picrate; it is probably a proteose, and it gives the Sakaguchi reaction for guanidine derivatives. Hypertensin requires an activator to produce its powerful peripheral vasoconstrictor effect. Owing to a constricting effect on the efferent glomerular arterioles, renin liberation can lead to a vicious cycle in the ischemic kidney. Mammalian renin does not react with the hypertensinogen of birds, fish, or reptiles, and vice versa, avian renin is inactive with mammalian hypertensinogen. Crystalline pepsin can form another pressor polypeptide (pepsitensin) from the hypertensinogens of various species.

Repeated injections of renin, or hypertensin, show decreasing effects (tachyphylaxis). This refractory state is induced by exhaustion of the activators and, perhaps, by formation of antipressor inhibitor substances. Since healthy kidney tissue destroys renin, the hypertension produced by a single ischemic kidney is usually temporary. Neurogenic factors play an important role in aggravating moderate hypertension of humoral origin. Administration of thiocyanates is an empirical treatment of hypertension; the blood thiocyanate level should not exceed 12 mg. per cent, in order to avoid toxic effects. Antirenin sera and the renin and hypertensin inhibitors manufactured by the kidney would seem to have greater therapeutic possibilities. Renin inhibitor extracts of kidney administered by mouth are effective in reducing hypertension. The kidney, blood plasma, and other tissues contain a specific hypertensinase, whose optimum pH is about 8. Tyrosinase preparations inactivate renin and hypertensin; the intravenous administration of tyrosinase lowers the blood pressure of hypertensive dogs. The ischemic kidney of the cat actively decarboxylates injected dopa to the pressor amine, hydroxytyramine, with production of acute renal hypertension. Intravenous *l*-dopa causes a marked rise in blood pressure in hypertensive patients, but not in normal men. Ischemia interferes with renal destruction of pressor amines by amine oxidase. This enzyme inactivates both hypertensin and hydroxytyramine; when injected it lowers the blood pressure of hypertensive dogs.

Decrease of blood pressure by hemorrhage or shock causes liberation of renin from the canine kidney. Experiments on hemorrhagic dogs suggest that exhaustion of hypertensinogen may be a cause of the fatal collapse of blood pressure following severe hemorrhage. Injection of a relatively small hypertensinogen fraction of ox plasma protein can restore the blood pressure in these animals. Nephrectomized rats exhibit a greater post-hemorrhagic fall in blood pressure than do normal animals.

During the last trimester of pregnancy, the conditions known as *pre-eclampsia* and *eclampsia* sometimes develop. Edema, albuminuria, and hypertension are prominent symptoms; they may be accompanied by cerebral, visual, gastro-intestinal, and renal pathology. The renal plasma flow is apparently normal in the hypertensive diseases of pregnancy. The renal symptoms are not usually traceable to pre-existent chronic glomerulonephritis or nephrosclerosis. In eclampsia, the blood uric acid is elevated and the alkali reserve is lowered. The plasma proteins tend to be low, and the protein intake may be inadequate. Hemoconcentration occurs prior to the eclamptic convulsions. The metabolism of hormones in eclampsia is considered on page 687.

AMINO ACIDURIA

Excessive excretion of amino acids is dependent on the elevation of the blood amino acid level, as in acute toxic necrosis of the liver, wasting diseases, and protracted fevers. Tyrosine and leucine crystals have, at times, been observed in the urine of patients with severe liver necrosis. The tendency toward increased histidine excretion, which occurs after the first month of pregnancy, has been attributed to inhibition of hepatic histidase by gonadotropic hormones.

ALCAPTONURIA

This is a rare disorder which is inherited as a recessive mendelian characteristic. It is reported that normal subjects kept on diets very low in ascorbic acid excrete homogentisic acid after tyrosine ingestion, but ascorbic acid therapy does not affect the hereditary condition. When alcaptonuric urine is made alkaline, or exposed to air, it turns dark brown or black, owing to oxidation of the homogentisic acid. The urine reduces alkaline copper reagents, but the homogentisic acid is optically inactive, does not form an osazone and is not fermented by yeast. The excretion of homogentisic acid is increased by a high protein diet and by the ingestion of phenylalanine or tyrosine. Alcaptonuria is a life-long condition. Pathological symptoms are absent in early life, but after midlife alcaptonuria is often accompanied by hypertrophic arthritis, and slowly developing *ochronosis* (melanotic pigmentation of cartilages, tendons, and fibrous tissues). Ochronosis can also result from long continued phenol application. The darkening of the urine, which frequently follows the administration of phenolic drugs, should not be confused with alcaptonuria.

CYSTINURIA

This hereditary condition occurs more frequently than alcaptonuria. It may be associated with malnutrition, pathological deposition of cystine in the tissues of infants, and formation of cystine calculi in the urinary tract.

Formation of cystine calculi is inhibited by the administration of alkali, since cystine is more soluble in alkaline urine. As much as 1.8 gm. cystine may be excreted daily in cystinuria. The urinary cystine is chiefly of endogenous origin; it is excreted (at least partially) as a labile precursor of unknown composition, which liberates cystine on standing. When cystine is ingested by cystinuric patients as the free amino acid or as glutathione, it is well utilized and its sulfur is excreted as sulfate; but administered methionine and cysteine are excreted largely as cystine. Methionine is, therefore, regarded as the precursor of much of the cystine excreted in this condition. A high protein diet seems to facilitate the utilization of methionine. Abnormal quantities of leucine, lysine, tryptophane, tyrosine, cadaverine, and putrescine have occasionally been detected in cystinuric urine.

INDICANURIA

Increased indican and phenol excretion results from the intestinal putrefaction which accompanies intestinal obstruction, paralytic ileus, peritonitis, achlorhydria, hypochlorhydria, duodenal ulcer, carcinoma of the liver, obstructive jaundice, typhoid fever, and constipation. Indican excretion can also be increased by bacterial decomposition of tissue protein, as in gangrene, extensive suppuration, empyema, tuberculosis, etc. Indicanuria is of little clinical interest.

MELANURIA

Melanin excretion in the urine is usually the result of extensive melanoma. Melanuria is present in approximately one fifth of the cases of melanotic sarcoma, usually after extensive metastasis has occurred. The excess melanin produced by these tumor cells is deposited in the skin. The pigment is usually excreted as a reduced colorless melanogen. As the urine stands in contact with air, the melanogen is reoxidized and the urine turns brown or black. At times melanin is found in the freshly voided urine. In the hereditary condition termed albinism, the normal formation of melanin by tissues is inhibited. This abnormality is a recessive mendelian characteristic.

PHENYLKETONURIA

The condition of mental deficiency known as imbecillitas phenylpyruvica, or phenylpyruvic oligophrenia, is inherited as a recessive mendelian characteristic. The patients exhibit amentia and extrapyramidal disturbances. The phenylpyruvic acid, which is excreted in the urine in this disease, arises from incomplete metabolism of phenylalanine. It gives a green color with ferric chloride solution. Blood does not contain phenylpyruvic acid, but phenylalanine is present in concentration of 28 ± 13 mg. per cent, whereas it cannot be detected in normal blood. Phenylketonuria

represents a failure to convert phenylalanine to tyrosine; instead, the kidney deaminates the phenylalanine to phenylpyruvic acid. Cerebral oxidation of glucose is subnormal in phenylpyruvic oligophrenia, and in mongolian idiocy.

TYROSINOSIS

This abnormality of tyrosine and phenylalanine metabolism rarely occurs in adults. The more common tyrosinosis of premature infants is relieved by administration of ascorbic acid. In tyrosinosis, the oxidation of tyrosine is inhibited and the amino acid is excreted in the urine, together with considerable *p*-hydroxyphenylpyruvic acid and small quantities of 3,4-dihydroxyphenylalanine. Phenylalanine, tyrosine, and protein given *per os* increase the excretion of these metabolites, which give Millon's reaction.

CREATINURIA AND MUSCULAR DISEASES

Excessive excretion of creatine is usually due to incomplete retention of creatine by muscles. It is generally accompanied by elevation of serum creatine. Physiological creatinuria in normal persons has been discussed on page 442. Pathological creatinuria occurs during the course of such muscle diseases as amyotonia congenita, amyotrophic lateral sclerosis, myasthenia gravis, myositis (especially myositis fibrosa), dermatomyositis, myotonia atrophica, progressive muscular dystrophy, trichinosis, progressive muscular atrophy secondary to lesions of motor neurones, atrophy from disuse, and the muscular rigidity of paralysis agitans. It is not present in myotonia congenita. In fact, patients having the latter disease show abnormal retention of administered creatine.

The *myotonias* are characterized by delayed relaxation and persistent contraction (fibrillation) of striated muscle. The abnormal muscle fibers are unusually sensitive to potassium ions, and exhibit fibrillary activity. There is an atrophic form (myotonia atrophica or myotonic dystrophy) and a hypertrophic type (myotonia congenita or Thomsen's disease). Congenital myotonia has been discussed on page 252. Myotonia appears at times in hypothyroidism. Fibrillation and rapid atrophy of muscle, with depletion of phosphocreatine, glycogen, and potassium, follow *denervation*. The atrophy may be due to exhaustion from rapid fibrillation. Unlike the fibrillation caused by tetanus toxin, that following denervation is not solely hypercholinergic in nature, since it is not arrested by curarine. Quinine diminishes the sensitivity to acetylcholine and potassium ions in denervation atrophy. *Progressive muscular dystrophy* is a familial disease which begins in early life, and is inherited through a sex-linked recessive factor. It is five times as frequent in males as in females. There is a primary hypertrophy of muscle fibers, followed by degeneration and atrophy, and replacement of muscle by fat and fibrous tissue. Hence, the adenosine triphosphate and phosphocreatine content of the affected

muscle is low. *Amyotonia congenita* is a congenital disease; the symptoms begin during the first year of life. The muscles are weak and small, but true atrophy does not occur. In myositis fibrosa, myasthenia gravis, familial periodic paralysis, progressive muscular dystrophy, and the nutritional muscular dystrophy due to vitamin E deficiency, decrease of muscle phosphocreatine has been demonstrated. In the last-named condition the muscle glycogen, acid-soluble phosphorus, creatine, nitrogen, magnesium, and potassium are reduced; muscle cholesterol, sodium chloride, and calcium are increased; and the muscle collagen is more than twice the normal concentration.¹ The wasted muscles in myotonia atrophica and progressive muscular dystrophy show similar changes, which are indicative of replacement of muscle fibers by connective tissue and lipides. The dystrophic muscles of vitamin E-deficient animals exhibit high oxygen consumption.

Daily administration of from 30 to 40 gm. glycine causes only slight excretion of creatine in normal persons, but in cases of progressive muscular dystrophy, myasthenia gravis, and amyotonia congenita it stimulates marked, temporary creatinuria. Prostigmine and ephedrine do not have this effect. After several weeks of continued glycine administration, creatinuria may be abolished almost entirely. As creatinuria diminishes, the creatinine excretion and the ability to retain creatine are increased. When glycine causes considerable creatinuria in primary myopathies, the patients may show temporary improvement. Glycine and prostigmine are generally ineffective in progressive muscular dystrophy. (See page 252 for a discussion of the use of prostigmine, quinine, etc., in cholinergic muscular diseases.) Oral administration of α -tocopherol abolishes creatinuria in nutritional muscular dystrophy, and it cures this experimental disease. Administration of vitamin E has also been reported to be beneficial in primary fibrositis. While the vitamin itself has little therapeutic value in human muscular dystrophies, its inositol ether is reported to be effective.

Creatinuria results from starvation, diabetes mellitus, severe liver necrosis or carcinoma, low carbohydrate diets, scleroderma, familial periodic paralysis, varied nervous diseases, congestive heart failure, hyperthyroidism, and thyroid therapy. All these conditions are accompanied by decreased glycogenesis in the muscles. Iodine medication temporarily decreases the creatinuria of hyperthyroidism, but not that which results from thyroxine administration. The weakness of hyperthyroid patients is related to muscular changes resembling those occurring in progressive muscular atrophy, and to impaired phosphocreatine metabolism. Creatinuria also occurs in phlebitis profunda, during menstruation and pregnancy, after castration, and, at times, after fractures or

¹ Deposition of excess collagen in the skin is a prominent feature of the degenerative condition called scleroderma. The skin becomes rigid and indurated, and usually it undergoes pigmentation and atrophy. Muscular pathology and creatinuria usually accompany scleroderma. Scar tissue, keloids, and fibromas also contain excess collagen.

amputation. Postpartum creatinuria is not due to involution of the uterus, since it is not abolished by hysterectomy; it is more closely related to lactation. The creatinuria of castrated animals is temporarily diminished by injection of testosterone.

INCREASED PROTEIN OF CEREBROSPINAL FLUID

Cerebrospinal fluid protein rises when the permeability of the choroid plexuses is increased, as by inflammatory conditions of the brain, cord, or meninges. Euglobulin and fibrinogen enter the subarachnoid space only during severe inflammation. Approximate estimates of the cerebrospinal fluid globulin concentration (Noguchi, Nonne-Apelt, Pandey, Ross-Jones, and tryptophane [Levinson] tests) have been employed clinically, but exact quantitative determination of the protein is preferable. In early stages of meningitis, the cerebrospinal fluid protein level may at times be normal, but in suppurative meningococcic, pneumococcic, streptococcic, and tuberculous meningitis and in acute syphilitic meningitis it rises to from 125 to 1,300 mg. per cent. In syphilitic meningitis, the spinal fluid usually shows a positive Wassermann reaction and abnormal colloidal gold curves which are functions of the albumin: globulin ratio. Abnormality of colloidal gold curves is usually dependent on an increased γ -globulin content of the spinal fluid. In the Froin syndrome, associated with compression of the spinal cord and retention of fluid in a cul-de-sac, very high protein concentrations are found. Specimens of fluid obtained from above and below the obstruction may show very different protein concentrations and thus assist the diagnosis of the Froin syndrome. Elevation of the protein to 200 mg. per cent is occasionally encountered in acute anterior poliomyelitis.

In non-infective meningeal irritation, and in serous meningitis resulting from influenza, mastoiditis, otitis media, pneumonia, typhoid fever, or uremia, the protein of cerebrospinal fluid tends to be near normal (20 to 70 mg. per cent). Cerebrospinal fluid protein often rises slightly, and temporarily, as the result of convulsions (epileptic seizures, spasmophilia in children, and uremic convulsions). It may be increased moderately by organic disease of the brain and cord, regardless of meningeal pathology, as, for example, in brain abscess, brain tumor, central nervous system lues, epidemic encephalitis, and cerebral hemorrhage, thrombosis, or embolism.

CHEMISTRY OF IMMUNITY, HEREDITY. AND DEVELOPMENT

"It is only as a last resort that we modify (as little as possible) the old ideas."
MORRIS R. COHEN

IMMUNE REACTIONS

The complex metabolic processes which generate resistance and immunity to infectious agents and foreign proteins are phases of endogenous protein metabolism. Foreign proteins are typical *antigens*, or substances whose parenteral administration to animals induces the formation of specifically modified serum globulins, termed *antibodies*. When a small quantity of ovalbumin is added to serum, *in vitro*, no significant reaction ensues; but the parenteral injection of a solution of ovalbumin into a living animal leads to a gradual accumulation of specific antiovalbumin protein in the plasma. This antiovalbumin is a globulin which will combine, *in vitro* and *in vivo*, with ovalbumin, but not with other proteins.

In suitable concentrations, the antibodies act as specific *precipitins*. For example, casein is flocculated as a casein-anticasein complex, when mixed with its specific antibody. The precipitin reactions are very useful for identifying foreign proteins and the infectious agents which contain them, also for the detection of antibodies in sera, and for the medicolegal identification of blood stains. When the antigen is contained within a foreign cell, the specific serum antibody frequently acts as an *agglutinin* to clump or agglutinate the cells by a precipitin reaction at their surfaces. In similar fashion, an antibody can function as an *opsonin* to change the surfaces of specific bacterial cells in a manner which sensitizes them to phagocytosis.

The visible results of an antigen-antibody reaction thus depend on physicochemical factors which are not essential to the specific immune reaction itself. The particle size of the immune precipitate determines its visibility. Dilute salt solutions are necessary for the flocculation of antigen-antibody compounds in precipitin and agglutinin tests; 0.85 per cent sodium chloride solution is usually employed for this purpose. Ten to twenty per cent sodium chloride reverses the immune reaction, and dissociates the specific precipitate into its antigen and antibody components. Agglutination occurs over a pH range of 3.7 to 9.0.

Under suitable conditions, an antibody can induce permeability changes in the membranes of specific cells which lead to their lysis or destruction. In this case, the antibody is operating as a *lysin* and, when erythrocytes are the substrates, as a *hemolysin*. A complex globulin component, found in the fresh serum and plasma of many animals, is necessary for specific lysis. This *complement* or *alexin* is a non-specific reactant, and its concentration in the plasma (0.8 per cent of the serum protein in man) is not increased in immunized animals. It produces no lysis until the cells are altered by combination with their specific antibody (in this instance, termed the *amboceptor* or *sensitizer*). Antigen-antibody complexes in the cell surfaces, or in the immune precipitates, unite chemically and irreversibly with complement. Lysis occurs after this combination has taken place at the cell surface. Complement is destroyed at temperatures above 50° C. Complement consists of an endpiece (α -globulin glycoprotein, with iso-

electric point at pH 6.35), a midpiece (β -globulin or euglobulin, with isoelectric point at pH 5.3), a phospholipide associated with the midpiece, and a fourth component which is apparently a glycoprotein. The β -globulin portion unites with the antigen-antibody compound.

Specific lysis is a convenient method for the detection of complement, and it is extensively used for the detection of syphilitic antibodies. In the *Wassermann reaction* the syphilitic antibody is first allowed to unite with an artificial "lipide antigen" prepared by alcoholic extraction of animal tissues. The resulting "antigen"-antibody compound combines with serum complement, and this complement-fixation interferes with the lysis of erythrocytes by their specific hemolysin. Non-specific hemolysis by snake venoms does not require the presence of complement; these hemolysins are lecithinases which act on phospholipide substrates to produce lytic lysophospholipides (page 189).

ANTIGENS

The majority of antigens are proteins, although certain polysaccharides are known to possess antigenic activity. Polysaccharides and proteins show striking chemical resemblances in their colloidal properties, chain structures, and polar acidic and basic radicals. Most native proteins are actively antigenic; the injection of a few γ of certain native proteins is sufficient to provoke antibody formation in small animals. Insulin, Warburg's yellow enzyme, and the crystalbumin of serum are rather ineffective antigens, and such low molecular protein preparations as albumoses, gelatin, histones, protamines, and proteoses have very feeble antigenic activities. However, proteins with low molecular weights can, at times, be transformed into very active antigens by coupling them with the substances called haptens (page 469). The lower peptides and the amino acids are entirely inactive. To function as an antigen, a protein must be capable of forming a suitable colloidal solution; thus, heat-coagulated proteins are antigenic only if they can be redissolved.

Pepsin digests antigens to small non-antigenic fragments. The latter do not give precipitin reactions with specific antibodies for the parent proteins, but they tend to react with antibodies for the corresponding metaproteins. The antigenicity of proteins is rapidly destroyed by dilute alkali, which racemizes the amino acid units and prevents digestion by enzymes. No single amino acid unit is responsible for the antigenic properties of protein; this is a function of the large intact molecule and its many peptide linkages. Plasteins, formed by the synthetic activity of proteolytic enzymes on protein degradation products, are antigenic; they also manifest a common, characteristic specificity.

Antigens which exhibit characteristic toxic effects in animals are termed *toxins*. They are classified as bacterial toxins, phytotoxins and zootoxins, according to their biological origin. The toxins are usually proteins of

comparatively low molecular weight, which are destroyed above 80° C., below pH 5, and above pH 9. With the exception of botulinus toxin and staphylococcic enterotoxin, the poisonous antigens are digested readily when taken orally. The highly specific manifestations of toxins may be classified roughly as neurotoxic actions, and endothelial poisonings which cause hemorrhage. Nerve tissue selectively removes neurotoxins from tissue fluids; in non-susceptible animals, such toxins remain in the circulation for long periods. After they assimilate toxins, the tissues often undergo cloudy swelling and fatty or amyloid degeneration. Toxins which are destructive to leukocytes are termed *leukocidins*; they are formed by pneumococci, staphylococci, streptococci, and other micro-organisms. Tuberculin is an exotoxin, secreted by *Mycobacterium tuberculosis*. It is employed extensively in skin tests, for the detection of antituberculin. Similarly, in the Dick test for scarlet fever, and in the Schick test for diphtheria, the respective toxins are injected to determine the presence of antibodies. Diphtheria toxin has been crystallized as a heat coagulable protein; crystalline crotoxin, of rattlesnake venom, is a protein which exhibits lecithinase activity. The α -toxin of type A *Clostr. welchii* is a lecithinase which hydrolyzes phospholipide to phosphocholine and diglyceride. The molecular weights and the isoelectric points of several toxins are given in Tables 71, page 397, and 10, page 47, respectively.

Bacterial antigen preparations, in the form of killed bacteria, are employed prophylactically as *vaccines* to induce temporary acquired immunity to certain diseases. *Bacteriophages* and *viruses* are very high molecular nucleoprotein antigens which possess marked infectious properties (page 492).

Specificity of Antigens

The remarkable specificities of the immune reactions permit the detection of differences in the structure and composition of proteins; they also allow the animal body to set up specific defense mechanisms against micro-organisms and viruses. Insight into the complexities of immune reactions requires recognition of the fact that immunological specificity is always relative, owing to varying degrees of chemical similarity between antigens. Each protein has a characteristic immunological identity, but it also resembles certain other proteins. The more closely antigens are related chemically, the greater is the tendency for cross reaction between the specific antibody for one of the proteins and the non-specific, related or *heterophile* antigens. Each antibody reacts most readily with its specific or *homologous* antigen; the cross reactions usually require considerably larger concentrations of the heterophile antigens. Distantly related proteins do not show evidence of cross reactions.

There are striking phylogenetic relationships between natural antigens; for example, the serum proteins of man and the anthropoids are more closely interrelated than are the serum proteins of distant species. Other

proteins which show phylogenetic relationships are the plant proteins, globins, pepsins, egg and milk proteins, and the globulins of brain, muscle, and other organs. By contrast, the amyloids, catalases, insulins, keratins, lens globulins, thyroglobulins, and flavoproteins of different species are less differentiated; their small species differences are overshadowed by a powerful radical common, in all species, to each of these proteins. Specificity of tissue proteins increases (and tolerance to transplants decreases) phylogenetically with ascent of the taxonomic scale, and ontogenetically during the development of the individual. The various proteins in an individual animal have no common immunological pattern, detectable by cross reactions; as a group, they are unable to provoke *autoimmunization*, or the production of antibodies, when reinjected into the same animal.

The interrelations of antigens are due partly to the chemical patterns of the proteins themselves; they are also tremendously affected by combination with prosthetic radicals, such as polysaccharides, lipides and polypeptides, and by the introduction of chemical radicals by oxidation (to oxyprotsulfonic acids), nitration (to colored xanthoproteins), halogenation (to iodoproteins and bromoproteins), coupling with diazonium compounds (to colored azoproteins), denaturation (intramolecular rearrangement), methylation, acetylation, sulfonation, reaction with ninhydrin, and so forth. However, the reaction of free amino radicals with formaldehyde or with nitrous acid, and their acetylation with ketene ($\text{CH}_2=\text{CO}$), cause little change in the specificity of proteins; frequently these reactions destroy the toxic activities of toxins and convert them into therapeutically valuable antigens termed *toxoids* or *anatoxins*. Such detoxicated antigens can still unite specifically with the original antitoxins; and, when injected, they provoke active immunization. About one third of the free amino nitrogen of diphtheria toxin (present chiefly in the lysine units) combines with formaldehyde during the production of toxoid. Sodium ricinoleate and bile salts can partially detoxify such toxins as ricin, diphtheria toxin, and tetanus toxin; they also modify living bacteria to form vaccines, but the chemical reactions involved have not been elucidated.

Cross reactions and related specificities of antigens do not always conform to phylogenetic relationships, nor are they exclusive functions of antigens with infectious or toxic properties. The only prerequisite for a cross reaction is that the antigens should be related chemically. Thus, iodoproteins cross-react with antibodies for bromoproteins; also, colored xanthoproteins and azoproteins, which evidently possess similar quinoid arrangements in their substituted tyrosine units, show interrelations.

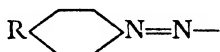
In addition to a dominant specificity, each antigen exerts less powerful immunological influences through secondary chemical radicals. Thus, the serum proteins of man and of anthropoids possess a common dominant specificity, but they differ in secondary respects. The injection of human serum protein into an anthropoid incites the formation of an antibody

sensitive to radicals which are not common to the serum proteins of the two species. Antisera for anthropoid serum protein are flocculated by the heterophile human serum protein; a more specific antibody for anthropoid protein can be detected in the filtrate. Each antibody is, therefore, adapted to a specific chemical influence or *antigenic determinant*. It is obvious that the injection of any ordinary protein, which has a varied assortment of chemical radicals, will incite the formation not only of one dominant antibody but also of several less effective antibodies related to secondary chemical radicals. Ordinary protein preparations frequently are mixtures, as demonstrated for caseins, clupeins, hemoglobins, serum proteins, etc.

HAPTENS

Polysaccharides, lipides, drugs, and certain ordinary chemical reagents can unite with proteins to produce greatly modified *complex* or *complete antigens*, whose specificities differ markedly from those of the parent antigens. These prosthetic radicals or haptens are important determinants of the serological specificity of complex antigens, especially when the haptens contain acidic, basic, or other polar radicals. A given hapten can unite with a variety of biologically unrelated proteins to produce a group of complex antigens which are endowed with a new dominant specificity, referable to the hapten. For example, the introduction of iodine atoms into the tyrosine units of a protein confers a dominant specificity, which is shared by other iodized proteins. The differing structures of the parent proteins are not destroyed, but the original immunological responses are masked or overshadowed by the formation of antibody for the new and powerful chemical radical. The diiodotyrosine unit determines the immunological similarities of various thyroglobulins, and a lipide hapten is thought to be responsible for the similarities of lens proteins.

Coupling of proteins with diazonium compounds has proved valuable in studies of hapten effects. By this reaction, the radical



is attached to tyrosine and histidine units of antigens. Each azoprotein shows cross reactions with antibodies for other azoproteins, and also with antibody for the parent protein. The azoprotein compounds of sulfonamides show interrelations and cross reactions. By various substitutions at —R of the above radical, it has been shown that polar radicals such as —CONHR, —SO₂NHR, —COOH, —OH, and —NH₂, are very active in immune reactions.

Studies with azoproteins have proved that space arrangements in antigens and haptens are important for immunological specificity. By coupling with diazonium compounds *d*-, *l*-, and *meso*-tartaric acid radicals have been linked to a protein antigen; the specific antibodies for the *d*- and

l-derivatives cross reacted with antibodies for the *meso*-antigen, but not with antibody for the enantiomorphic derivatives. Individual amino acid radicals attached to proteins in similar fashion impart new characteristic specificities. Only those pairs of derivatives which contain added glycine or alanine, leucine or valine, or aspartic or glutamic acids have similar immunological characteristics; the amino acids of these pairs are also chemically similar. Terminal amino acids which have free carboxyl radicals are most important in serological specificity, whereas the peptide linkages are concerned with antigenicity.

Glycoproteins and nucleoproteins constitute the major portion of the natural antigens extractable from bacteria. These antigens are rich in haptens which react powerfully and specifically with the antibodies for the natural bacterial proteins; the haptens also tend to produce toxic effects when injected into specifically infected animals. A mixture of antigen and hapten is not sufficient to produce a complex antigen; actual chemical combination must occur, either *in vitro* or as the result of enzyme activities in cells. Type III pneumococcus polysaccharide hapten has been combined chemically with serum globulin to produce a complex antigen which can incite the formation of an antibody specific for type III pneumococci.

Type Specificity and Polysaccharide Haptens

Haptens are influential determinants of the strain characteristics of any particular cell. It is common knowledge that human sera contain antibodies, called *isohemagglutinins*, which can cause either agglutination or lysis of transfused erythrocytes. The isohemagglutinins are inherited as recessive characteristics. The four most important immunological strains of human erythrocytes are classified as blood groups I or AB, II or A, III or B, and IV or O. Cells of these groups differ in their content of two important polysaccharide haptens, A and B, which are inherited as dominant mendelian characteristics. Hence, non-paternity can occasionally be proved through blood types. These haptens, or *agglutinogens*, arise in the developing fetus during the second month, whereas the isohemagglutinins, or antibodies, appear from the third to the twelfth month after birth. The isohemagglutinins in the serum of any particular individual are specific for the haptens *not* present in his erythrocytes. Blood group determinations are made prior to transfusion except when plasma from blood banks is used; the erythrocytes and sera of patient and donor are also cross matched, inasmuch as the four principal blood groups are gross classifications of hundreds of smaller immunological variations. Haptens A and B have been found in body cells other than erythrocytes; such differentiations may be partly responsible for certain failures in skin grafting and in tissue or tumor transplantation. Polysaccharide hapten A (Table 81) has been prepared from salivary and gastric mucins, urine, and commercial peptone and pepsin. The hapten B polysaccharide has been isolated from blood,

saliva, and gastric juice. Blood group haptens M, N, and Rh are also found in erythrocytes and occasionally in other tissues, but not in saliva. Normal human serum does not contain isohemagglutinins for these groups, and the M and N groups have little significance for transfusion. The isohemagglutinin for the Rh factor, and its relation to erythroblastosis fetalis, are discussed on page 551.

Many bacterial species are similarly subdivided into types or strains. Specific polysaccharide haptens have been reported for meningococcus, Friedländer's bacillus, *Aer. aerogenes*, *N. gonorrhoeae*, *H. influenzae*, *P. vulgaris*, *Br. melitensis*, *B. anthracis*, *Mycob. tuberculosis*, *Mycob. leprae*, *E. typhosa*, *Esch. coli*, *Sh. dysenteriae*, *S. paratyphi*, *S. enteritidis*, *V. cholerae*, *C. diphtheriae*, *Clostr. welchii*, *Pasteurella* and *Phytomonas* genera, *Rickettsiae*, *Ascaris lumbricoides*, spirochetes, yeasts, molds, and the like. The principal strain characteristics of pneumococci, salmonella, staphylococci, and streptococci are determined by polysaccharides. There are fundamental differences in the carbohydrate metabolism and hapten content of rough (R), smooth (S), and other bacterial variants. The virulent smooth colonies elaborate the larger quantities of polysaccharides, and they contain type-specific toxic haptens which are not found in the rough colonies. The R strains, and non-capsulated forms of bacteria, contain chiefly the species-specific polysaccharides which are less important factors in virulence. Virulent S forms can be converted into attenuated R forms by cultivation in type-specific immune sera. The O or somatic antigens and the corresponding antisera for R and S types are obviously different. Micro-organisms are thus able to synthesize a mosaic of complex antigens, which include the R, S, and H (flagellar) antigens. In general, the R haptens and protein antigens determine phylogenetic relationships, while S polysaccharide and lipid haptens are determinants of type specificity.

The bacterial polysaccharides are associated prominently with capsules, but they are also secreted as gums or slimes. The importance of the polysaccharide bacterial haptens was established through studies of pneumococci, whose capsules contain large quantities of type-specific polysaccharides. At times, free capsular polysaccharides appear in the blood and urine of patients with severe pneumonia. In type III pneumonia, the lungs may contain as much as 1.2 to 1.5 per cent of the specific polysaccharide. The chemical composition of these high molecular thermolabile haptens is shown in Table 80. Types II, III, and VIII pneumococcal polysaccharides are interrelated through their cellobiuronic acid units. Antiserum for a synthetic cellobiuronic derivative (an azoprotein) has been shown to cross react with types II, III, and VIII pneumococcal polysaccharides, and to protect mice against infection by these strains of pneumococci. Similar relations exist between type II pneumococcal polysaccharide and antisera for a gentiobiuronic acid azoprotein. Soil bacteria, when grown in the presence of types II or III pneumococcal polysaccharide as the sole source of carbon, develop adaptive enzymes which

hydrolyze these polysaccharides specifically, dissolve the capsules from the corresponding strain of organisms, and relieve animals infected with the bacteria (page 90). Sulfanilamide and sulfapyridine also affect the formation of capsules by pneumococci; their inhibitory activity can be overcome by addition of *p*-aminobenzoic acid to the culture medium. The pneumococcal type can be changed by inoculating a small quantity of an R strain into a medium which contains a desoxyribonucleic acid preparation made from an S strain of another type. Conversion of type I to types II and III pneumococci, and of type II to types I and III, has been accomplished in this manner.

TABLE 80

TYPE SPECIFIC PNEUMOCOCCAL POLYSACCHARIDES

TYPE	UNITS
I . . .	<i>d</i> -Galacturonic acid, acetyl- <i>d</i> -glucosamine (in aldobionic acid linkage)
II . . .	<i>d</i> -Glycuronic acid, <i>d</i> -glucose (in cellobiuronic acid linkage)
III . . .	<i>d</i> -Glycuronic acid, <i>d</i> -glucose (in cellobiuronic acid linkage, the cellobiuronic acid units joined in 1, 3 linkage)
IV . . .	<i>d</i> -Glucose, acetylated amino sugar
VIII . . .	<i>d</i> -Glycuronic acid, <i>d</i> -glucose (partly in cellobiuronic acid linkage)
XIV . . .	<i>d</i> -Galactose, acetyl- <i>d</i> -glucosamine

Types I, IV, V, XII, XIX, and XXV contain the largest quantities of nitrogen; twenty-three of the types give Ehrlich's test for glucosamine, while eleven are probably nitrogen-free.

Types V, VII, XI, XII, XX, XXV, XXX, XXXIII, and XXXIV have the largest concentrations of acetyl radicals (united to amino sugars); many types also contain esterified phosphoric acid radicals.

Types I, II, III, VIII, IX, XII, XXII, XXV, XXVII, and XXXIII give positive tests for uronic acids.

Type V reduces alkaline copper reagents without hydrolysis.

Type VI and type XXVI pneumococci are identical, and type XV and type XXX polysaccharides are closely related.

The molecular weight of type I pneumococcal polysaccharide is $150,000 \pm 50,000$; of type III polysaccharide, 62,000; and of type VIII polysaccharide, 140,000.

Type specificity of polysaccharides has now been demonstrated in a great variety of cells, including bacteria, fungi, protozoa, and yeasts, and also in viruses. The composition of some of these polysaccharides is given in Table 81.

The stereoisomerism of carbohydrates permits a great variety of specific prosthetic polysaccharides, whose uronic acid and amino sugar units exert prominent serological influences. Mucins contain similar polysaccharides, and they probably play a physiological role in the immunity of the gastrointestinal tract. When gastric mucin is introduced into the peritoneal cavity with typhoid organisms, it enhances the pathogenicity and inhibits the phagocytosis of the typhoid bacilli. The *lysozymes* (low molecular enzymatic proteins of egg white and of the mucous secretions of animals) lyse a variety of bacteria, and hydrolyze certain bacterial mucoids and

TABLE 81

MISCELLANEOUS POLYSACCHARIDE HAPTENS

HAPTEN OF	UNITS
<i>B. anthracis</i>	<i>d</i> -Galactose, acetyl- <i>d</i> -glucosamine
<i>C. diphtheriae</i>	Aldobionic acid, <i>d</i> -arabinose, <i>d</i> -galactosamine, <i>d</i> -galactose
Erythrocytes, group II	<i>d</i> -Galactose, acetyl- <i>d</i> -glucosamine
<i>E. typhosa</i> , type II, somatic or O antigen	<i>d</i> -Galactose, <i>d</i> -glucose, <i>d</i> -mannose
Friedländer's bacillus, type A	<i>d</i> -Glucose, <i>d</i> -glycuronic acid (in aldobionic acid linkage)
Friedländer's bacillus, type B	<i>d</i> -Glucose, aldobionic acid
Friedländer's bacillus, type C	<i>d</i> -Glucose, aldobionic acid
<i>H. influenzae</i>	Pentose, uronic acid
<i>Mycob. tuberculosis</i> ¹	<i>d</i> -Arabinose, <i>d</i> -galactose, inositol, <i>d</i> -mannose
<i>S. typhimurium</i>	<i>d</i> -Galactose, <i>d</i> -glucose, <i>d</i> -mannose
<i>Ser. marcescens</i> ²	Acetylhexosamine, aldohexose, methylpentose, phospholipide
<i>Sh. dysenteriae</i>	<i>d</i> -Galactose, <i>l</i> -rhamnose, acetyl- <i>d</i> -glucosamine
<i>Str. haemolyticus</i> , type A	<i>d</i> -Glycuronic acid, acetyl- <i>d</i> -glucosamine
<i>Str. pyogenes</i> , groups A and C	Hyaluronic acid
<i>V. cholerae</i> , type I	<i>d</i> -Galactose, <i>d</i> -glycuronic acid, acetylaminohexose
<i>V. cholerae</i> , type II	Arabinose, <i>d</i> -galactose, <i>d</i> -glycuronic acid, acetylaminohexose
<i>V. cholerae</i> , type III	<i>d</i> -Glucose, acetylaminohexose

¹ The molecular weight of *Mycob. tuberculosis* polysaccharide is 7,300; that of *Mycob. leprae* is 2,500.

² This polysaccharide causes hemorrhage and necrosis in mouse tumors.

polysaccharides. Avidin preparations show lysozyme activity, and biotin apparently increases the action of lysozymes.

Spreading Factor

Certain strains of *Clostridia*, staphylococci, pneumococci, and other micro-organisms produce the enzyme *hyaluronidase*, which is a diffusion or spreading factor. Hyaluronidase occurs also in snake and insect venoms, and in mammalian connective tissue and testis. The enzyme increases the permeability to invasive infections and toxins of living and dead tissues, but not of recently injured tissue areas. The spreading of bacteria is made possible by hydrolysis of hyaluronic acid compounds (mucins) in the mesenchymal intercellular cement of the connective tissues. Sperm hyaluronidase facilitates penetration of the cervical mucus plug and of the ovum jelly. The enzyme hydrolyzes hyaluronic acid conjugated with

protein, and its action is inhibited by its antibody, serum globulin, heparin, chondroitin-sulfuric acid, and gastric mucin. The appearance of the enzyme in a streptococcus culture causes disappearance of the capsules, since hyaluronidase destroys those capsular polysaccharides which contain hyaluronic acid. Addition of potassium hyaluronate to *Clostr. welchii* cultures stimulates the production of adaptive hyaluronidase.

Cross Reactions Induced by Polysaccharide Haptens

Haptens are responsible for many heterophile phenomena (cross reactions of antibodies with phylogenetically unrelated antigens) (Table 82). Perhaps the most extensive cross reactions are those induced by Forssmann's "heterophile antigens," which are chemically related polysaccharide haptens present in many avian, piscine, and mammalian tissues, pneumococci, green streptococci, typhoid bacilli, the hemorrhagic septicemia group, the *Salmonella*, *Sh. dysenteriae*, *B. anthracis*, *N. catarrhalis*, and *Clostr. welchii*. The Forssmann polysaccharides are chemically related to blood group A hapten. They endow various proteins with a common immunological behavior. Since Forssmann haptens do not occur in the

TABLE 82

AGGLUTININ AND PRECIPITIN CROSS REACTIONS

ANTIBODY FOR	VS.	HAPTEN OF
<i>R. prowazeki</i>		<i>P. vulgaris</i> , type X ₁₉
Pneumococcus, types I and III		Gum acacia (galacturonic acid in aldobionic acid linkage)
Pneumococcus, types I and XIV		Blood group A
Pneumococcus, type II		Brewer's yeast polysaccharide, vegetable gums, and Friedländer's bacillus, type B
Pneumococcus, type III		Gonococcal and meningococcal polysaccharides
<i>H. influenzae</i> , type B		Pneumococcus, type VI
<i>E. typhosa</i>		<i>S. enteritidis</i>
<i>S. schottmülleri</i>		Blood group A
<i>Sh. dysenteriae</i>		All blood groups
Enteric organisms		Red yeast
<i>R. dermatroxenus</i>		<i>R. orientalis</i>
Snake venom		Various snake and scorpion venoms
Mocassin venom		<i>S. typhimurium</i>
Pneumococci		Streptococci (protein hapten)
<i>Mycob. tuberculosis</i>		Diphtheria (lipide hapten)

The colon-typhoid group shows cross reactions within the group, because of the various proteins.

Arachnolysin, a plant protein, agglutinates human and rabbit erythrocytes, but not horse or guinea pig erythrocytes.

tissues of cattle, deer, pigs, rats, rabbits, or most primates, these species form the Forssmann antibody most readily. Some of the tissue polysaccharides are extractable by alcohol, and have been classified erroneously as lipide haptens.

Lipide and Polypeptide Haptens

The serological importance of lipide haptens was suggested by the Wassermann and other complement-fixation reactions, the Forssmann heterophile antigens, and the lecithinase activities of snake venoms. Pure lecithin or cephalin cannot replace alcoholic tissue extracts in either the Wassermann or Forssmann reactions. The beef heart phospholipide which is active in the Wassermann test is a polysaccharide-containing lipide. The lipide haptens concerned in all of these reactions, as well as those present in *Mycob. tuberculosis*, *Mycob. leprae*, and related micro-organisms, contain polysaccharide units. The phospholipides of human tubercle bacilli have two polysaccharide fractions, which contain *d*-mannose and glycerol, and *d*-mannose and inositol, respectively. These characteristic "lipide antigens" are, therefore, fatty acid esters of polysaccharide haptens. Simple lipides have little hapten activity. The ordinary sterols and, to a smaller extent, lecithin and cephalin, precipitate or inactivate immune sera by non-specific chemical combination.

Important polypeptide haptens have been discovered. The capsules of the anthrax bacillus and of *B. mesentericus* contain a unique polypeptide, which consists of from 40 to 50 units of *l*-glutamic acid (the unnatural isomer, of the *d*-series). This polypeptide resists the digestive action of proteolytic enzymes and is not easily destroyed in mammals.

In conclusion, it should be stated that not all prosthetic radicals of proteins are either type- or species-specific haptens; this is illustrated by the action of the Forssmann haptens. The prosthetic polysaccharides of serum proteins are not type-specific, and glycogen and chondroitin-sulfuric and hyaluronic acids have no demonstrable hapten activity. Neither does heme impart a dominant specificity to the hemoglobin molecule. The heme prosthetical radical can be changed chemically, as in methemoglobin, cyanhemoglobin, and carbonylhemoglobin, without affecting specificity. Non-specific prosthetic substances do not inhibit the immune reactions of the antigens which contain them. A true polysaccharide hapten inhibits the reaction of the corresponding complete antigen with its antibody.

CHEMOTHERAPY

The synthetic *sulfonamides* (sulfanilamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfapyridine, sulfasuxidine, sulfathiazole, etc.) possess polar radicals, which are capable of uniting with certain bacterial proteins *in vivo*. These drugs are bacteriostatic, not bactericidal; they inhibit

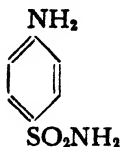
bacterial growth by competing with natural coenzymic growth stimulants, thereby causing metabolic alterations which affect the production of bacterial proteins and polysaccharides.

p-Aminobenzoic acid, and local anesthetics derived from it, can nullify the chemotherapeutic activity of the sulfonamides, and restore the growth of certain micro-organisms. Sulfonamide-resistant strains of staphylococci produce more *p*-aminobenzoic acid than susceptible strains. Because methionine antagonizes the effects of small concentrations of sulfonamides on certain bacteria, it has been suggested that these drugs inactivate a bacterial enzyme which has a *p*-aminobenzoic acid coenzymic radical and is essential for growth and the synthesis of methionine. Prolonged feeding of the poorly absorbed sulfaguanidine to rats suppresses their growth by inhibiting bacterial production of *p*-aminobenzoic acid and other vitamins in the intestine. Sulfanilamide inhibits the oxidation of *p*-aminobenzoic acid by peroxidase; and both *p*-aminobenzoic acid and the sulfonamides inhibit phenol oxidases.

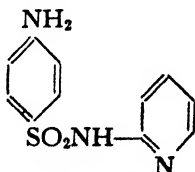
The sulfonamides appear to counteract several coenzymes, since purines and cozymase preparations can antagonize sulfonamide bacteriostasis of such bacteria as *L. casei*. Sulfapyridine inhibits the growth response to nicotinic acid in canine black tongue, and it prevents transformation of this vitamin to nicotinamide nucleotide in certain micro-organisms. Sulfanilamide is a caryotoxic agent (page 210); it inhibits synthesis of iodo amino acids by thyroid tissue; and it also inactivates carbonic anhydrase and causes acidosis and loss of body base. The latter effect is not inhibited by *p*-aminobenzoic acid, and it is not produced by other sulfonamides.

Since the discovery of the effects of sulfonamides, it has been shown that other sulfonic acid derivatives can block specific enzyme activities. Thus, α -aminosulfonic acid analogues of natural amino acids, pyridine-3-sulfonic acid, and pantoyltaurine inhibit the growth of certain bacteria by competing with essential amino acids, nicotinic acid, and pantothenic acid, respectively. Pantoyltaurine can protect rats against streptococcal infections. The remarkable chemotherapeutic activities of sulfonamides may be attributed to their competitive action as analogues of essential coenzymic metabolites. They block the activities of certain enzymes, and are themselves of no specific value to cell economy.

SULFANILAMIDE

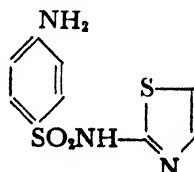
*p*-Aminobenzenesulfonamide

SULFAPYRIDINE



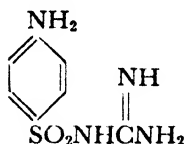
2-Sulfanilamidopyridine

SULFATHIAZOLE



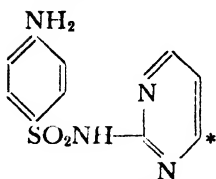
2-Sulfanilamidothiazole

SULFAGUANIDINE OR
SULFANILYLGUANIDINE



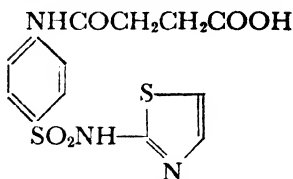
p-Aminobenzene-
sulfonylguanidine

SULFADIAZINE



2-Sulfanilam-
idopyrimidine

SULFASUXIDINE OR
SUCCINYLSULFATHIAZOLE



2-Succinylsulf-
anilamidothiazole

SULFAMERAZINE is methylsulfadiazine, with the CH_3 radical at the position marked by an asterisk.

Sulfanilamide, sulfadiazine, and sulfamerazine are especially effective for treating infections caused by meningococci, pneumococci, and streptococci, while sulfathiazole is most effective against gonococci, staphylococci, *Clostridia*, *Str. viridans*, and most urinary tract infections. Certain strains of the organisms mentioned resist sulfonamide treatment, and some become sulfonamide-fast during inadequate therapy. These drugs are ineffective in the presence of pus. Sulfonamide chemotherapy has proved beneficial in other infections, such as actinomycosis, chancroid, *H. influenzae* infections, trachoma, and undulant fever, but it is of little aid in anthrax, colds, diphtheria, influenza, malaria, plague, rheumatic fever, subacute bacterial endocarditis, syphilis, trench mouth, tuberculosis, typhoid, fungus infections, Rickettsial diseases, or virus diseases (except lymphogranuloma venereum).

The sulfonamides named above are used locally on wounds, burned areas, and ulcers, as powders or in ointments, to prevent gas gangrene and similar infections. They may also be implanted intraperitoneally for the treatment of peritonitis. Slowly absorbed sulfonamides (sulfaguanidine and sulfasuxidine) are employed for certain intestinal infections (acute bacillary dysentery and ulcerative colitis). They suppress *Esch. coli* and cause a marked decrease in Gram-negative fecal organisms.

The dosage of a readily absorbed sulfonamide is about 0.1 gm. per kg. of body weight the first day, with subsequently decreased doses to maintain optimal effective blood levels (7 to 15 mg. per cent). Small quantities of sulfonamides can induce persistently high blood levels during renal insufficiency; relapse of the infection may then occur in the presence of high blood sulfonamide concentrations, owing to progressive inactivation of the drugs through acetylation. Blood sulfonamides are partially bound to plasma albumin. They can be determined colorimetrically in deproteinized blood filtrates by N-(1-naphthyl)-ethylenediamine dihydrochloride after diazotization. Acetylsulfonamides do not react with the reagent unless they are first hydrolyzed by acid.

These drugs must be administered with care, since excessive dosage and abnormally high blood levels may cause skin eruptions, febrile reactions, hemolytic anemia, leukopenia or agranulocytosis, methemoglobinemia or sulfhemoglobinemia, and renal complications due to crystallization of the less soluble acetyl derivatives. It is important to maintain the urinary output at 1200 to 1500 ml. daily, in order to minimize urinary crystallization of acetylsulfonamides. Sulfapyridine is the most toxic of the sulfonamides listed, and its intestinal absorption is erratic. Sulfathiazole enters the cerebrospinal fluid with difficulty. Since the placenta is readily permeable to sulfonamides, the period of therapy should be limited for pregnant women. Prolonged administration of sulfaguanidine to rats reduces the growth rate; produces deficiencies of *p*-aminobenzoic acid, biotin, and vitamin K; and causes bone marrow aplasia, enlargement of the thyroid gland, and sclerosis and calcification of blood vessels (page 664). Biotin and folic acid deficiencies result from prolonged sulfasuxidine feeding. Folic acid, pyridoxin, and certain liver preparations ameliorate the agranulocytosis and anemia of sulfonamide poisoning.

Microbial Antibiotic Agents

As mentioned on page 471, soil bacteria can produce adaptive enzymes which hydrolyze specifically the capsular polysaccharides of pneumococci, and destroy the capsules of these micro-organisms. Like antipneumococcic sera, they decrease the virulence of the pneumococci and render them susceptible to phagocytosis. The soil bacillus, *B. brevis*, is a widely distributed aerobic organism which produces typical antibiotic agents of non-enzymic character. Gramicidin and tyrocidine, isolated as crystalline polypeptides from cultures of this bacterium, are remarkably bacteriostatic to Gram-positive micro-organisms *in vitro*. About 45 per cent of the amino acid units in gramicidin, and 15 per cent of those in tyrocidine, are unnatural *d* isomers. The polypeptides are therefore resistant to ordinary proteolytic enzymes. Gramicidin is a neutral polypeptide, insoluble in water but soluble in alcohol. Its *d* amino acid units are leucine and valine; the composition of this antibiotic agent is given in Table 69, page 383. Tyrocidine is a basic polypeptide which contains *d* phenylalanine units; it combines readily with proteins, and is inhibited by them. Tyrocidine and gramicidin are detergents which are bacteriostatic *in vivo*, but they are too toxic, hemolytic, and leukocytolytic to be used parenterally. When given orally, their bacteriostatic and toxic properties are greatly diminished. Preparations of these antibiotic substances have been used locally for the treatment of abscesses, burns, ulcers, and superficial infections.

Phospholipides counteract the antibacterial action of gramicidin and other surface-active detergents and wetting agents. Anionic detergents (alkyl sulfates, fatty acids, bile acids) and basic dyes affect chiefly the Gram-positive group of bacteria, while cationic detergents (quaternary

nitrogen bases) are bactericidal to both Gram-positive and Gram-negative micro-organisms. Protamines can sensitize Gram-negative bacteria to the action of substances which ordinarily affect only Gram-positive organisms. The Gram-positive characteristic is attributable to the presence of magnesium ribonucleate in combination with protein of the cytoskeleton; it can be abolished by culturing bacteria in magnesium-deficient media.

Since the discovery of gramicidin and tyrocidine, numerous antibiotic agents have been prepared from *Actinomyces*, *Aspergilli*, *Penicillia*, and other micro-organisms (Table 83). *Actinomycins* exhibit marked activity against bacteria and fungi *in vitro*, but they are toxic to animals and afford little protection against infection. *Streptomycin* and *streptothricin* are antibiotic to fungi, yeasts, and bacteria. Streptothricin is a polypeptide which causes elongation of bacterial cells, but it exhibits delayed toxicity in animals. Streptomycin is a relatively non-toxic compound of streptidine (a cyclic guanidine derivative) and streptobiosamine (a methylamino disaccharide). One mg. of crystalline streptomycin is equal to 1,000 S units; its antibiotic activity is inhibited by cysteine. It is poorly absorbed from the gastro-intestinal tract, and is excreted in the urine after parenteral injection. Streptomycin is detectable in the blood stream as long as 6 hours following intravenous injection. The placenta is permeable to streptomycin, while only small quantities enter the cerebrospinal fluid. This antibiotic agent is particularly useful in combating Gram-negative bacteria. It may be given orally in intestinal infections; intramuscularly or subcutaneously in systemic and urinary tract infections, tularemia, or Friedländer bacillus infections; and intrathecally in influenzal meningitis. Results of therapy in tuberculosis and typhoid fever are equivocal at present. The suggested parenteral dosage is 250,000 units every 6 hours.

Of the antibiotic substances produced by *Aspergilli*, aspergillic acid, clavacin, fumigacin, fumigatin, and especially gliotoxin are toxic when injected in animals; flavicin has little toxic effect. Fumigacin and fumigatin are moderately active antibiotic substances. Gliotoxin and clavacin (clavatin, claviformin, or patulin) are both fungistatic and bacteriostatic; the action of clavacin on Gram-negative bacteria is inhibited by sulfhydryl compounds.

The *Penicillia* produce less toxic and very effective antibiotic agents. *P. notatum* forms an enzymatic flavoprotein (penicillin B, notatin, or penatin), whose bacteriostatic action is the result of hydrogen peroxide liberation during oxidation of glucose to gluconic acid (page 96). Citrinin and penicillic acid are slightly bacteriostatic; the action of the latter on Gram-negative bacteria is inhibited by sulfhydryl compounds. Acrylophenone is a synthetic bacteriostatic and fungistatic agent, which has the β -unsaturated ketone radical common to several of these antibiotic agents (Table 83).

TABLE 83
ANTIBIOTIC SUBSTANCES

SUBSTANCE	CHEMICAL NATURE	GRAM TYPE CHIEFLY AFFECTED	SOURCE
Acrylophenone	Aromatic ketone ¹	both	Synthetic
Actinomycin A	$C_{45}H_{58}N_{10}O_{11}$ (Polycyclic pigment)	positive	<i>Act. antibioticus</i>
Aspergillid acid	Pyrazine derivative ¹	both	<i>Asp. flavus</i>
Chetomin		positive	<i>Chaet. cochliodes</i>
Chlorellin		both	<i>Chlorellae</i>
Citrinin	Heterocyclic compound ¹	both	<i>P. citrinin, Aspergilli</i>
Clavacin (clavatin, claviformin, patulin)	Heterocyclic compound ¹	both	<i>Asp. clavatus, Gymnoascus, Penicillia</i>
Flavacidin		positive	<i>Asp. flavus</i>
Flavicin	Acid	positive	<i>Asp. flavus</i>
Fumigacin (helvolic acid)	$C_{26}H_{44}O_8$	positive	<i>Asp. fumigatus</i>
Fumigatin	Quinone ¹	both	<i>Asp. fumigatus</i>
Gigantic acid			<i>Asp. giganteus</i>
Gliotoxin	Indole derivative ¹	positive	<i>Asp. fumigatus, Gl. fimbriatum, Penicillia, Trichoderma</i>
Gramicidin	Polypeptide	positive	<i>B. brevis</i>
Iodinin	Phenazine pigment ¹		<i>Chromob. iodinum</i>
Penicillid acid	Unsaturated keto acid ¹	both	<i>P. cyclopium, P. puberulum</i>
Penicillin	Thiazolidine acid ¹	positive	<i>P. notatum, P. chrysogenum, Asp. flavus</i>
Penicillin B (notatin, penatin)	Flavoprotein	both	<i>P. notatum</i>
Puberulic acid	$C_8H_8O_6$	positive	<i>Penicillia</i>
Pyocyanine	Phenazine pigment ¹	both	<i>Ps. aeruginosa</i>
Spinulosin	Quinone ¹		<i>P. spinulosum, Asp. fumigatus</i>
Streptomycin	$C_{21}H_{37}N_7O_{12}$	both	<i>Actinomyces</i>
Streptothricin	Polypeptide	both	<i>Actinomyces</i>
Tyrocidine	Polypeptide	both	<i>B. brevis</i>
Violacein	Pigment	positive	<i>Chromob. violaceum</i>

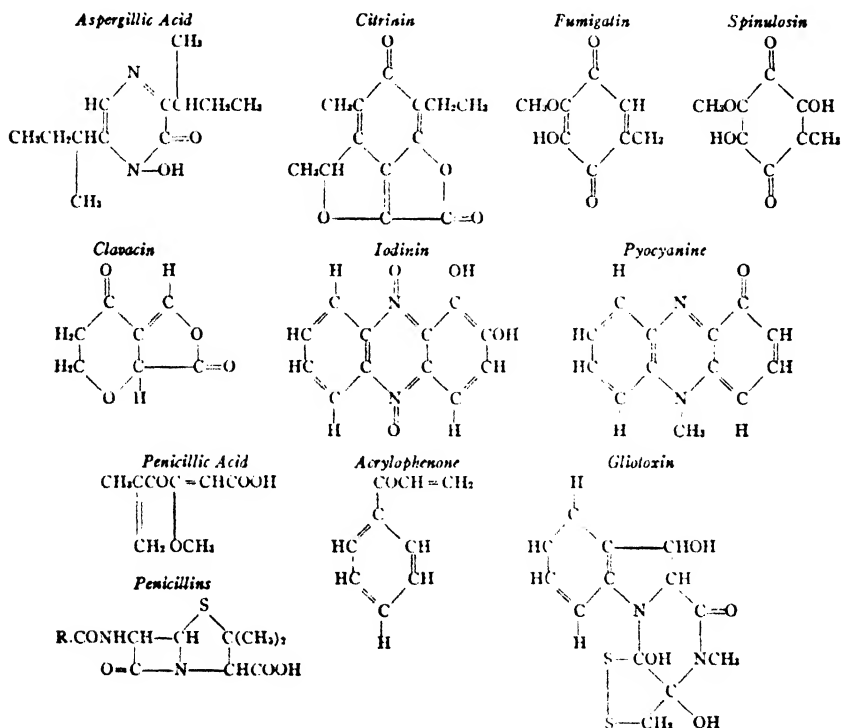
¹ Formulae are given on page 481.

The most important non-toxic antibiotic agent is *penicillin*, prepared from *P. notatum*. Penicillin is an acid which is soluble in organic solvents; its salts are water soluble. The crystalline sodium salt has an activity of one international unit per 0.6γ, but the pharmaceutical preparations are less active and quite impure. Penicillin is destroyed by heat, acid, alkali, or exposure to oxidizing agents. The several known varieties of penicillin (F, G, K, and X) have a $CH_3CH_2CH=CHCH_2$, benzyl, *n*-heptyl, or *p*-hydroxy-benzyl radical, respectively, at R of the formula (page 481).

Penicillin is bacteriostatic to a variety of Gram-positive micro-organisms, also to gonococci and meningococci, and in higher concentrations to *Treponema pallidum*. It is ineffective against most Gram-negative organisms, mycobacteria, malaria organisms, fungi, yeasts, and viruses. The several strains of a given micro-organism differ in their susceptibility to penicillin, and a few can become penicillin-resistant in patients as the result of inadequate therapy. Penicillin is more than a thousand times as bacteriostatic as sulfonamides. Its action is not inhibited by pus, and it is effective against sulfonamide-fast bacteria. The bacteriostatic action of penicillin is related to suppression of cell division and formation of giant and involution forms, followed by autolysis (or by phagocytosis in animals).

Penicillin has given remarkable therapeutic results in *Clostridia*, gono-

coccal, pneumococcal, staphylococcal, and streptococcal infections, meningitis, osteomyelitis, infected wounds, ocular and mastoid infections, and anthrax; and it is of value in actinomycosis, syphilis, typhus, and yaws. It is relatively ineffective in colds, dysentery, pemphigus, pertussis, rheumatic fever, subacute bacterial endocarditis, trachoma, tuberculosis, tularemia, typhoid fever, ulcerative colitis, undulant fever, and infections with animal parasites. The susceptibility of an infectious organism to penicillin can be determined by measuring the effect of the antibiotic



agent on the growth of a laboratory culture. Certain micro-organisms (*Esch. coli*, the paracolon bacillus, etc.) produce an enzyme, penicillinase, which inactivates penicillin, but this enzyme is not usually responsible for the development of penicillin resistance. Penicillinase activity is inhibited by azide, iodoacetate, and sulfhydryl compounds.

Oral administration of penicillin is very inefficient because of destruction by the acid gastric juice, inactivation by intestinal bacteria, and other factors. It is absorbed much more readily from the duodenum or muscle than from the rectum, subcutaneous tissues, or body cavities. It does not enter erythrocytes, cerebrospinal fluid, tears, or saliva readily,

but the placenta is permeable to penicillin. Penicillin combines with serum albumin. Intramuscular or intravenous injection is the preferred method of administration; the dosage depends on the type and severity of the infectious process. As in sulfonamide therapy, it is important to provide an effective initial dose in order to avoid the development of bacterial resistance. Usually, 15,000 to 20,000 units of penicillin in normal saline solution are given intravenously, followed by 5000 units at hourly intervals; or 250,000 to 600,000 units are used daily in a continuous intravenous saline drip. When injected intramuscularly, 10,000 to 20,000 units are administered every two to four hours. Accessory intrathecal or intracisternal injections of 10,000 units once or twice daily are used in meningitis. About 2,000,000 units are given during an eight day period for the treatment of primary syphilis. For local therapy, 30,000 to 40,000 units are injected twice daily into empyema cavities; and penicillin is used as a powder, spray, wet dressing, or ointment for burns and wounds. Penicillin administration is continued until clinical improvement, negative bacterial cultures, and normal body temperature become manifest.

Penicillin is excreted so rapidly by the renal glomeruli and tubules that the blood stream is practically cleared of this substance within 2 hours after its parenteral injection. When given intravenously, about 60 per cent appears in the urine; some of the remainder is inactivated in unknown fashion in the body. Esters of penicillin have no antibiotic effect *in vitro*, but are effective in animals, even when given orally; they are slowly hydrolyzed to free penicillin by the tissues. Occasionally penicillin therapy causes urticaria or fever, owing to impurities in the commercial preparations.

ANTIBODIES

The circulating antibodies are modified serum globulins; their formation, during immunization, frequently causes an increase in the γ -globulin concentration of the serum. Like normal serum globulin, the antibody globulins do not act as foreign proteins, and the *active immunity* induced by infection is relatively permanent. Antibodies to injected foreign immune sera are formed readily, and they are serologically identical with the antibodies for the normal serum globulin of the same foreign species. The *passive immunity* afforded by injection of foreign antiserum is temporary, because these antibodies are foreign serum globulins, which soon undergo the immune reaction. The disappearance of such antibodies coincides with the production, by the host, of antibody for the foreign serum globulin.

Diphtheria antitoxin has been crystallized as a protein with a molecular weight of 90,500. Other pure antibodies have been separated from serum by precipitation with specific polysaccharide haptens. Types I and III pneumococcal antibodies of cow, horse, and pig sera are globulins, with

molecular weights near 930,000, while all investigated antibodies of monkey, rabbit, and human sera (and also horse diphtheria antibody) have molecular weights near 185,000. The molecular weights of the normal serum globulins of the horse and rabbit are near the latter figure (Table 71, page 397). Rabbit antiserum is most efficient in the treatment of meningitis, since the relatively low molecular weight of the antibody allows better penetration of membranes. Antibodies are destroyed by temperatures above 55° C. by heat denaturation; they are rapidly digested by pepsin, less readily by trypsin. Many of the properties of antibodies and of enzymes are similar, owing to their protein structure; but antibodies do not act as enzymes in immune reactions. Certain antigens, such as snake venoms and the toxin of type A *Clostr. welchii*, have separate enzymatic and antigenic activities (pages 58 and 189).

The immune specificities of antibodies are undoubtedly relative, just as antigenic specificity is relative. To associate each antigen with a single unique antibody would require an amazing array of individual serum globulin components in normal individuals, who can be relatively immune to a great variety of infectious agents. The antibodies found in men and animals are, at times, so paradoxical as to be impossible of explanation on the basis of previous exposure to specific antigens. It is reasonable to assume that antibodies, like antigens, tend to be polyvalent and to unite with more than one antigen through their several chemical radicals.

Two biological classifications of antibodies are recognized: *natural* or *congenital antibodies*, which are normal cellular proteins formed under genetic influences; and *acquired antibodies*, which are synthesized in response to infection or to the parenteral introduction of foreign proteins (active immunity). The latter type of antibody can be introduced by antitoxic serum therapy, to create temporary passive immunity during infections.

Studies with labeled antigens, such as arsenical proteins and chromoproteins, indicate that antibodies do not contain the dominant specific radicals of the corresponding antigens. The amino acid distribution of purified pneumococcal antibody is practically identical with that of normal serum globulin of the same species; but during immunization, the newly formed serum globulin is modified by reorientation of its amino acid units.

Formation of Antibodies (Immunization)

The formation of antibodies depends on structural differences between the antigenic protein and the proteins of the host. The underlying metabolic process should not be regarded as arising, *de novo*, on contact of living cells with foreign protein, but rather as a modification of normal protein synthesis in cells. This plastic distortion of the normal synthetic process can be induced either by a foreign protein, or by the complex antigen formed by union of a hapten with normal tissue protein. Biological protein synthesis follows very exact patterns; in cells, the amino acids are

selected and arranged with mathematical precision under the influence of the normal protein templates which are duplicated during the synthesis. The cell proteins are, in other words, coenzymic templates which specifically direct the synthetic reactions. The presence of a foreign protein distorts this duplicating mechanism and small amounts of a modified serum globulin, with a definite chemical relation to the antigen, are formed.¹ One molecule of antigen elicits the formation of numerous molecules of antibody globulin. The antigen continues to induce unnatural orientation of amino acid units until some secondary metabolic process disposes of the foreign protein. It has been shown that the injection of an antigen can cause the appearance of one hundred times its weight of antibody in the blood, in addition to considerable quantities in the tissues. However, even at the height of immunization, the antibodies usually constitute less than 1 per cent of the total serum protein.

Antibodies can be formed, *in situ*, by many animal and plant cells; they can also be formed in tissue cultures. There is much evidence to suggest that the reticulo-endothelial cells are the chief sources of the circulating antibodies of animals. In mammals, the liver is probably an important site of antibody synthesis, since this organ is one of the chief sources of normal serum globulins. Hemorrhage accelerates the synthesis of normal serum globulins, and it stimulates antibody production. The synthesized antibodies enter the blood in small quantities and are gradually assimilated by the tissues, which retain them for varying periods. Several days after a single injection of antigen, the antibody appears in the blood; it attains a maximum concentration in a week, and disappears gradually over a period of weeks to years. The ultimate fate of the antibodies is not known. Like the normal tissue proteins, they can undergo hydrolysis; they also exchange their chemical radicals without hydrolysis. Administration of amino acids containing N¹⁵ shows that one half of the nitrogen in circulating pneumococcal antibody can be replaced within twelve days, even though the quantity of the antibody is decreasing at the time. In contrast to active antibodies, injected passive antibody does not undergo transamination, and it is removed more rapidly from the circulation (about 50 per cent in two days).

Caseins and lens proteins, which normally are not in intimate contact with the circulation, can act as iso-antigens to produce *iso-antibodies* in the same species. It is possible that certain pathological alterations of tissues may allow the formation and liberation of sufficiently modified cellular protein to stimulate auto-immunization; if so, the iso-antibodies produced would probably accumulate in the specific organs containing the degenerated proteins. The injection of kidney or muscle proteins into

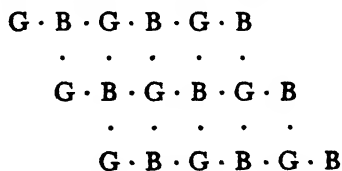
¹ One theory considers antibody as a serum globulin whose polypeptide chain ends are adapted to the configuration of the antigen. Some evidence has been presented by the proponents that denaturation of serum globulin in the presence of an antigen, with unfolding of the polypeptide chain, allows the globulin to act as an antibody.

another species causes the formation of organ-specific antibodies. When such antisera are introduced into the species from which the antigens were obtained, they cause degeneration of the specific organs. Typical nephritic and nephrotic lesions have been produced by the injection of antikidney sera. (See page 551 for a discussion of iso-immunization in erythroblastosis fetalis.)

The Antigen-Antibody Reaction

The combination of antigen with antibody is a colloid chemical reaction of marked specificity. Specific flocculates produced in the immune sera adsorb appreciable quantities of salts and lipides, but they do not contain non-specific proteins. Adsorptive and electrostatic phenomena are merely accessory factors to the specific chemical immune reaction, as illustrated by the following experiment. In separate, identically buffered solutions, crystalline ovalbumin and horse serum albumin are mixed with purified antibodies for horse serum and ovalbumin, respectively. No precipitate appears in either mixture. When a small portion of one of the mixtures is added to the other, a specific flocculate appears, despite the constant electrostatic conditions and practically identical isoelectric points of the crystalline antigens and of the two serum globulin antibodies.

The reaction of the polar radicals of antigen and antibody is similar to that of inorganic ions during crystal formation. The immune reaction can be reversed by dissociation of the specific compound into its antigen and antibody components below pH 4.5, above pH 9.5, in from 10 to 20 per cent sodium chloride solution, or by warming. The antibody is also liberated by tryptic digestion of the antigen. Maximal fixation of antibody at the surfaces of antigenic cells occurs near the isoelectric point of the serum employed. Since films of globular antigens and antibodies can form immune compounds, the gross shape of the molecule is not concerned. Quantitative studies have shown that an antigen (or polysaccharide hapten) and its specific antibody are polyvalent. Hence, the initial compound continues to add molecules of either substance, depending on the proportions of the reactants present. The immune precipitate may be represented as a lattice structure, as follows:



In this schematic diagram, G indicates the antigen or polysaccharide hapten, and B, the specific antibody. The final dimensions of the particles are limited by the relative concentrations of the reactants and by the insolubility of the growing particles. Excess of antigen gives a product which

contains relatively more G and tends to be soluble, owing to limitation of lattice growth from lack of B. The valency of antigens for antibodies tends to increase with the molecular weight of the antigen; thus, more than one hundred molecules of antihemocyanin can combine with one molecule of hemocyanin. It can readily be appreciated that toxin-antitoxin flocculates may exhibit either toxic or antitoxic properties, according to the quantities of each constituent in the lattice. The composition of such immune compounds depends more on the relative proportions of the components than on their individual concentrations. When the two reactants are present in the proportion necessary for optimal flocculation, the high molecular antibody globulins constitute from 80 to 98 per cent of the specific precipitates. Polyvalency of toxin is responsible for the well-known *Danysz phenomenon* (i.e., the quantity of antitoxin sufficient to neutralize toxin when added *in toto* is insufficient when added by portions).

Considerable heterophile antigen is required to form an insoluble immune compound by cross reaction, probably because in these cases the polar linkage with the antibody is less stable and the product is more highly dissociated. The reaction of antigen with antibody is not the rigid lock and key reaction postulated by Ehrlich, but it does conform to his criterion of a specific chemical reaction which requires definite chemical radicals and spatial configurations in the reactants. Small hapten molecules combine readily with specific antibodies, but the resulting compounds are insoluble only when the hapten is a colloid or is conjugated with a colloid. Otherwise, the compounds are soluble non-diffusible globulin complexes. By forming such compounds, low molecular haptens cause specific inhibition of precipitin reactions; these effects are useful in determining the nature of the active chemical radicals in an antigen. For example, both diiodotyrosine and thyroxine unite with antibodies for iodoproteins and inhibit flocculation of these antibodies by the iodoproteins. Recent experiments show that non-colloidal polyvalent haptens which contain several haptenic radicals per molecule can act as precipitins to specific antibodies and can fix complement in this fashion.

TISSUE IMMUNITY

The individual living cells possess the mechanism for antibody formation. Tissues can also assimilate circulating antibodies from the blood stream. Infections usually begin as localized inflammatory processes, which stimulate defense mechanisms in adjacent tissues. Even after the infectious organisms have gained access to the blood stream, they are abundantly exposed to the activities of endothelia and leukocytes. Hence, the circulating antibodies are only secondary factors in tissue immunity. Precipitin tests show that acquired circulating antibodies appear first in the liver and spleen, and are subsequently distributed to many parts of the body. After being assimilated by the tissues, they become fixed rather

firmly, and this portion is not readily available for combination with intravenously injected antigens. Antigen injected into a tissue of an immunized animal is largely fixed by combination with antibody at the site of injection, whereas in a non-immunized animal a larger proportion of the injected antigen enters the circulation and stimulates the formation of serum antibody.

The antibody which is present in the regenerated serum formed after bleeding an immunized animal is partly mobilized from the tissue store. Similar mobilization occurs during *anamnesis* (the temporary recall of tissue antibodies into the circulation by the injection of a non-specific protein, or of a quantity of the specific antigen too small to stimulate renewed immunization). Anamnestic mobilization is more rapid than the manufacture of serum globulin antibody. Anamnesis may be regarded as one object of parenteral non-specific protein therapy; another is the stimulation of leukocytosis. Non-specific anamnesis is the only effect of vaccine therapy during active infection.

Local tissue immunity persists much longer than humoral immunity, and antibodies can be found in the liver, spleen, and kidney after they have disappeared from the blood stream. The presence of antibodies in serum is, therefore, not strictly necessary for immunity, and the blood antibody level need not always parallel the state of immunity.

Antibodies unite with, but do not destroy, antigens and the cells which contain them. Injected protein antigens leave the blood stream slowly; they are partially transformed into protein which resembles the cellular constituents. A variable portion of the injected antigen is excreted in the urine. Injected ovalbumin disappears from the blood somewhat more rapidly than horse serum protein, but both can be detected in the plasma for as long as a week. Injected antigens labeled with arsenic, iodine, or pigments are distributed to the tissues and body fluids (especially to joint fluids, very little to cerebrospinal fluid). The major fraction accumulates in the liver and other reticulo-endothelial tissues.

By their opsonic action, the antibodies sensitize bacteria for eventual phagocytic disposal; and by their antitoxic effects, they minimize damage to the host's tissues and to the phagocytes. The pathogenic properties of bacteria depend partly on their toxicity to phagocytes, and their inhibition of phagocytosis and leukocytic digestion. For this reason, antitoxins are often better therapeutic agents than antibacterial sera. Sulfonamide or antibiotic therapy leads to modification of the unfavorable activities of bacteria.

Bacteria which cannot be lysed by serum complement require phagocytosis for their destruction. Leukocytosis is characteristic of most infections, and it is regarded as evidence of an active defense mechanism. Normally, there are $7,000 \pm 3,000$ leukocytes per cu. mm. of human blood (65 ± 5 per cent neutrophil polymorphonuclear cells, 29 ± 4 per cent lymphocytes, 4 ± 2 per cent monocytes, 2.5 ± 1.5 per cent eosinophils, and

0.4 ± 0.2 per cent basophils). The neutrophils increase markedly in many (but not all) infections, whereas in allergic conditions, worm infestations, and certain skin diseases, the eosinophils are relatively increased. After an infectious agent has gained local entrance into the body, inflammatory processes raise a temporary barrier to its spread. Polymorphonuclear (neutrophil) and eosinophil cells, derived from the bone marrow, move actively toward the inflammatory zone under the stimulus of leukocytosis-provoking factor, leukotaxine, foreign proteins, and nucleic acids (page 61). They engulf sensitized bacteria actively and destroy them by means of proteolytic enzymes. Even more important are the mononuclear phagocytes or macrophages, including the monocytes and tissue macrophages. These cells tolerate more unfavorable conditions and, hence, preponderate during the later stages of inflammation; they also phagocytize both bacteria and damaged host cells. Mere accumulation of phagocytic cells is not sufficient for successful phagocytosis of virulent organisms; the opsonic activities of antibodies are also important.

HYPERSENSITIVITY

Conditions of abnormal sensitivity to antigens and haptens are widespread in animals and man; they include anaphylaxis and the anaphylactoid allergies and idiosyncrasies. The cardinal symptoms of these hypersensitivities resemble those produced by the injection of histamine or proteoses. They are referable largely to stimulation of smooth muscle and to increased capillary permeability, with marked transudation of plasma proteins into the tissues. Adrenaline and ephedrine tend to counteract these effects, and they are used clinically to provide temporary relief from anaphylactoid symptoms. The combination of an antigen with its specific antibody *in vivo* induces rapid liberation from the tissues of a toxic *anaphylatoxin*, which is either histamine or a closely related substance. Histamine is regarded as the anaphylatoxin, even though certain organs and species appear to be resistant to its action, and heparin inhibits anaphylactic shock but not histamine intoxication. The anaphylatoxin originates from a tissue precursor (not from the provocative antigen). A most minute quantity of foreign protein can elicit anaphylactic shock in a sensitized animal. Excised tissues exhibit the same hypersensitivity as the intact animal. Anaphylatoxin, or histamine, is liberated most readily by the hypersensitive liver, lung, intestine, and kidney, and less extensively by hypersensitive brain, skin, and spleen, when the isolated tissues are incubated with the specific antigen.

Intravenous injection of proteoses produces symptoms almost indistinguishable from true anaphylaxis, namely, rapid liberation of histamine, temporary marked fall in blood pressure, stimulation of smooth muscle, increased transudation of serum proteins, fever, increased blood heparin concentration (resulting in incoagulability of plasma), increased excretion

of urinary nitrogen, and hypersecretion of lymph, milk, bile, and urine. The intravenous injection of a non-specific protein, or other foreign colloid, causes a somewhat different shock reaction characterized by rapid chill, fever, sweating, joint and muscle pains, increase in blood leukocytes, increased pulse rate and blood pressure, increased lymph flow, increased peristalsis, and mobilization of serum enzymes and tissue antibodies. Such non-specific parenteral protein therapy (usually milk, or typhoid vaccine) has been used empirically in the treatment of general sepsis, arthritis, skin infections, corneal ulcers, general paresis, gonorrhea, typhoid fever, encephalitis, and so forth.

Anaphylaxis

True anaphylaxis results from the parenteral introduction of a specific antigen into a sensitized animal. An initial injection of the antigen, even in exceedingly minute dosage, induces a state of hypersensitivity which develops gradually during a period of from one to three weeks. A second injection of the same antigen (or its corresponding hapten), following accumulation of the serum antibody, induces a violent anaphylactic response. The symptoms of experimental anaphylactic shock include marked depression of blood pressure, asphyxial convulsions, increased catabolism of proteins, edema, urticaria, hemorrhage, loss of complement, leukopenia, eosinophilia, decreased coagulability of the blood (due to hepatic liberation of heparin), and violent contraction of the bronchial muscles, uterus, stomach, intestines, and bladder. Human beings are less susceptible to these effects than are most laboratory animals; the chief symptoms of clinical anaphylactic shock are pruritis, urticaria, erythema, cyanosis, coughing, dyspnea, edema, weakness, and coma. The symptoms may appear immediately upon the injection of horse serum into patients of the "horse asthmatic" group. Repeated experimental anaphylactic shock in animals leads to degenerations of the heart, kidneys, liver, and lungs.

The hypersensitive state may continue for weeks, or even years; but immediately following anaphylactic shock, a temporary state of *desensitization* or antianaphylaxis develops. A practical therapeutic method of inducing desensitization is the repeated injection, at intervals of from three to seven days, of quantities of the antigen too minute to produce anaphylaxis. Replacing the blood of a sensitized animal by transfused normal blood does not desensitize the animal. Hypersensitivity can be induced occasionally in normal animals by the injection of sufficient serum from sensitized animals. The induced hypersensitive state appears only after a latent period, during which the transferred antibody is being fixed in the tissues. Any increase in the quantity of circulating antibody (as by the desensitization procedure mentioned above) protects temporarily against anaphylactic shock, by allowing antigen-antibody combination

outside the cells. When the immune reaction takes place intracellularly, the anaphylatoxin is liberated.

Local Anaphylactoid Phenomena

Sensitized tissues fix circulating antigens, and such tissues can be desensitized only temporarily. Local anaphylaxis occurs when the specific antigen is brought into contact with a tissue of a systemically sensitized animal, or when the tissue is first sensitized locally, and the antigen is then injected parenterally. In the first instance, the response, termed the Arthus phenomenon, consists of local inflammation, severe hemorrhage and edema, and local necrosis, which appear in several days. When the tissue is sensitized locally and the supply of circulating antibody is small, a much less drastic skin reaction develops, namely, a wheal with local erythema, as in the common skin tests. This reaction usually develops within one-half hour, and then disappears gradually.

The clinical *allergies* are defined as human hypersensitivities to ordinarily harmless substances. They represent more or less localized anaphylactoid states, in which tissue antibodies cause reactions varying from mild evanescent edema and erythema to severe tissue destruction. The local sensitivity may be associated with general immunity, or it may be independent of it. Allergic states are often initiated by previous exposure to specific antigens; but at times, they must be attributed to congenital tissue constituents, characteristic of the individual. The interpretation of all sensitivities as the result of previous specific immunization becomes untenable when one considers, for example, that human sera fail to lyse chicken erythrocytes, whereas duck and guinea pig sera lyse human erythrocytes. Such reactivities are endogenous, and have little to do with the foods of the animals. All types of human allergy show strong familial tendencies, which are at least partly due to congenital peculiarities of the tissue proteins. Hereditary allergy is known as *atopy*; patients with atopic types of allergy are difficult to desensitize.

The allergic condition termed *hay fever* is an inflammatory process of the upper respiratory tract. It is usually caused by pollen sensitivity and is, therefore, often seasonal in character. The odoriferous substances of flowers are also capable of acting as respiratory allergens. The symptoms respond less readily to adrenaline or ephedrine medication than do those of asthma or the skin allergies. Temporary preseasonal specific desensitization affords greater relief. The more permanent types of hay fever result from hypersensitivities to foods, drugs, animal danders, and house dust. Wheat, milk, and eggs are the most common provocative foods, although many others are individually responsible for certain cases. Similar specific sensitivities are the cause of *asthma*, a paroxysmal dyspnea from bronchial constriction, which is accompanied by a productive cough, due to edema of the bronchial mucous membranes. Allergens of house dust and of rag-

weed pollen are apparently glycoproteins. The antigens of dwarf and giant ragweed pollen which are responsible for hay fever and asthma have been isolated electrophoretically and named *artefolin* and *trifidin*, respectively. The complete ragweed antigens are compounds of proteins and flavonol glycosides.

Symptoms of *alimentary allergy* include edema of the gastro-intestinal tract, vomiting, diarrhea, and abdominal pain. Allergy can cause some forms of the periodic incapacitating headache, *migraine*, which results from cerebral vasodilatation. Contact of the specific antigen with sensitized gastro-intestinal or respiratory mucosae is sufficient to provoke symptoms in all of the allergic conditions. Foods can induce all types of allergy, and the successful treatment of the patient requires elimination of the specific substance from the diet. In sensitive children, casein hydrolyzate may be substituted for cow's milk.

The *skin allergies* include urticarias, eczemas, and certain angioneurotic edemas, which are caused by drugs, chemicals, cosmetics, animal furs, foods, and plant substances (including *urushiol*, the active component of poison ivy). Many occupational forms of dermatitis are included in this category. Intradermal skin tests find their most successful application in urticarial and erythematous allergies, and in the eczematous eruptive forms of drug allergy, while patch tests are more useful in studies of exanthematous types and of contact dermatitis. A positive skin test, following intradermal injection of an antigen, is indicated by occurrence of the wheal and erythema within a few minutes. Such immediate reactions occasionally result from the tuberculin test and from the Schick test for diphtheria. The usual response in these tests, as well as in the brucellosis test and the Dick test for scarlet fever, where toxins are injected, is a delayed skin reaction of the inflammatory type in non-immune persons, and little or no reaction in those who are immune. Only skin hypersensitivity of the immediate type can be transferred passively. The positive patch test, from antigen applied to the intact skin, consists of an infiltrated inflammatory area appearing in from one to three days. Many false positives are obtained in skin tests, because of cross reactions and multiple sensitivities; these may be considered as aggravating factors to the dominant sensitivity.

The *drug idiosyncrasies* represent haptenic immune reactions, usually manifested by skin allergy. Azodyes cause anaphylactoid symptoms when they are injected into animals sensitized with the corresponding azoproteins. Anaphylactoid drugs either combine with tissue proteins to form foreign antigens *in vivo*, or they cross react with normal tissue constituents in susceptible individuals. The typical anaphylactoid drugs contain such chemical radicals as aromatic nuclei, arsenic, or loosely bound nitro or halogen radicals, which combine readily with proteins. Antipyrine, arsphenamine, atropine, bromides, cocaine, novocaine, quinine, and salicylates are common causes of anaphylactoid symptoms. The hyper-

sensitive individual is usually affected by only one drug. Symptoms of pruritis, skin eruptions, edema, and urticaria appear after a latent period.

A condition known as *serum disease* occurs in certain patients who have received more than 100 ml. of foreign sera, parenterally. After an interval of from four to twelve days, fever, edema, skin eruptions, enlarged lymph glands, and polyarthritides appear; recovery occurs usually within a week. *Bacterial allergies* may occur during such infections as gonorrhea, pneumonia, rheumatic fever, syphilis, and tuberculosis. These allergic states are caused by sensitization to bacterial antigens, and they are responsible for some of the lesions and symptoms of infectious diseases. The hypersensitive state tends to persist for long periods after the infectious process has subsided. Hence, specific skin tests, including the tuberculin test, can cause local swelling, erythema, induration, and necrosis, both during and following the infection.

VIRUSES

This term is applied to infectious agents which pass bacteriological filters. Human diseases caused by filtrable viruses include chickenpox, the common cold, dengue, epidemic encephalitis, herpes, epidemic influenza, measles, German measles, mumps, infantile paralysis, psittacosis, rabies, smallpox, warts, and yellow fever. There are also numerous virus diseases of animals (cowpox, distemper, foot and mouth disease, hog cholera, rabbit papilloma, Rous sarcoma, etc.) and of plants (the mosaic diseases). Virus diseases are characterized by primary proliferation and degeneration of cells, and by secondary inflammation. The typical virus cannot be cultivated in lifeless media; but it is multiplied in living cells. Virus-infected cells frequently contain microscopic particles, the inclusion bodies; those of cowpox consist largely of nucleoprotein.

Viruses can be separated from ordinary tissue proteins by differential high speed centrifugation. Plant mosaic, equine encephalomyelitis and rabbit papilloma viruses have been isolated as nucleoproteins of very high molecular weight. Four strains of tobacco mosaic virus, two of cucumber mosaic virus, the tobacco necrosis and tomato bushy stunt viruses, and a nucleoprotein which exhibits poliomyelitis infective properties have been crystallized. Nucleoprotein viruses can be inactivated and disassociated to smaller units by urea solutions, or by exposing them to a pH below 2 or above 9. The virus of tobacco mosaic disassociates in alkaline solution into subunits which have molecular weights of 40,000 to 50,000. Viruses can be inactivated without degradation, by aging, by ultraviolet irradiation, and by x-rays; also by treatment with formaldehyde or nitrous acid, which produces toxoid-like proteins. When the amino radicals are restored to the formalized virus, its infectious activity returns. Digestion by pepsin causes a loss of activity proportional to the quantity of virus digested; the mosaic viruses are trypsin resistant, in contrast to the normal plant proteins.

The nucleic acid content varies from 4 to 40 per cent in different viruses; chicken tumor, equine encephalomyelitis, bushy stunt and tobacco mosaic viruses contain ribonucleic acids of higher molecular weight than those of yeast nucleic acid, although they contain similar chemical units. Influenza, rabbit papilloma, vaccinia, and psittacosis viruses contain desoxyribonucleic acids. These prosthetic radicals are bound more firmly in the virus proteins than are the nucleic acids in sperm nucleoproteins. The nucleoproteins of certain complex viruses are combined with polysaccharides; cowpox, influenza, equine encephalomyelitis, and Rous sarcoma viruses appear to contain considerable lipide. When the viruses are treated with phosphatase preparations, their nucleic acid radicals are removed by hydrolysis and infectivity is abolished. Tobacco mosaic virus is reversibly inactivated by crystalline ribonuclease, owing to complex formation. The protein of tobacco mosaic virus has very little glycine, histidine, lysine, or sulfur-containing amino acids. Neither the oxidation of sulfhydryl radicals nor the action of reducing agents destroys the activity of tobacco mosaic virus, but iodination of its tyrosine units causes inactivation. The several strains of the virus differ in their amino acid composition; only the rib-grass strain contains methionine.

Some virus particles are roughly spherical in shape, while others are fibrous or rodlike. Examination of the tobacco mosaic virus aggregates, in the electron microscope, demonstrates that their rodlike molecules are arranged in parallel fashion. Diameters of the several virus particles, as determined by various physicochemical methods, are as follows: psittacosis, 275 $m\mu$; cowpox, 200 $m\mu$; herpes simplex, 200 $m\mu$; rabies, 125 $m\mu$; influenza, 100 to 120 $m\mu$; chicken tumor, 70 $m\mu$; rabbit papilloma, 44 $m\mu$; yellow fever, 20 $m\mu$; poliomyelitis, 15 $m\mu$; and foot and mouth disease, 20 $m\mu$. Tobacco mosaic virus particles have a cross section of approximately 15 $m\mu$, and a length of 280 $m\mu$, as measured in the electron microscope. After combination with specific antiserum, the particles present a fuzzy appearance and they increase in size to 60 $m\mu$ by 300 $m\mu$. The bushy stunt and tobacco necrosis viruses are spherical particles with diameters of 26 $m\mu$ and 20 $m\mu$, respectively. The molecular weights of the viruses vary from such tremendous values as 8,500,000,000 for psittacosis virus, to 400,000 for foot and mouth virus (Table 71, page 397). The larger virus particles, therefore, attain the dimensions of certain living cells, while the smaller ones are exceeded in size by such protein molecules as urease, thyroglobulin, horse pneumococcal antibody, fibrinogen, myosin, nucleohistone, and the hemocyanins.

Nucleoprotein viruses are active antigens, and a number of virus infections provoke lasting immunity, probably because the viruses tend to persist in the tissues of the recovered host. Viruses which have been modified or attenuated by formaldehyde, phenol, ultraviolet light, and the like are used to produce immunity to such virus diseases as rabies and smallpox. The behavior of these nucleoproteins demonstrates that in-

fectivity is not a unique property of living organisms. The multiplication of virus nucleoprotein is an autocatalytic reaction, which evidently requires the organization and energy sources of living cells. It differs from the autocatalytic formation of pepsin and trypsin, in that the virus is not liberated from a precursor. Tobacco mosaic virus can infect immunologically unrelated plants (tobacco, nightshade, petunia, phlox, spinach, and tomato), whose differing tissue proteins could hardly be polymerized to form the same virus molecules. The viruses are, therefore, synthesized by cells. They act as templates for their duplication by cells, and they exert directive activities on protein syntheses which bear some resemblances to antigenic functions. The rate of virus multiplication tends to vary with the metabolic activity of the infected tissue; it is usually most rapid in young and growing tissues. Very small concentrations of cyanide retard the synthetic multiplication of tobacco mosaic virus in leaf cells. Since the cells cannot hydrolyze the virus protein readily, it continues to increase in quantity even when normal cellular proteins are being depleted by physiological processes. Nitrogen-deficient plants cannot use virus protein to replace normal proteins. The quantities of virus nucleoprotein in severely diseased tissues vary from 1 γ per cent to 500 mg. per cent. In rabbit papilloma infection, the virus may attain a concentration of 0.2 mg. per cent of the body weight of the animal.

It is possible that abnormal nucleoproteins are concerned in cancerous processes, but specific proteins for the usual clinical malignancies have not been found. Since most malignant abnormalities are not infectious, the activity of these hypothetical nuclear substances would more closely resemble the endogenous metabolism of the genes than the activities of viruses.

BACTERIOPHAGES

These are specific substances which cause the appearance of lytic plaques in bacterial cultures. Like the viruses, they are toxic and infectious; they do not respire and cannot be multiplied except by living cells. However, phage duplication differs from virus multiplication in that prophages have been detected in bacterial cells. Phage production is autocatalytic in nature, and resembles the conversion of pepsinogen and trypsinogen to pepsin and trypsin. In activated staphylococci, the formation of phage is inhibited by iodoacetate, and the phage precursor is inactivated photodynamically in the presence of methylene blue.

The phages accumulate in degenerating bacterial cultures; they are found in various animal tissues, and phages for enteric organisms are present in sewage. Dozens of different bacteria have been shown to liberate specific phages during degeneration. There are several phages for one bacterium, corresponding to the bacterial polysaccharide haptens with which the phages combine. These bacterial polysaccharides can inhibit phage activity. R and S strains form different phages; the antisera

for S strains show antiphagic activity. Mutation of phages occurs. Staphylococcus phage has been separated as a trypsin-resistant nucleoprotein. The molecular weights of the few phages investigated vary from 400,000 to 300,000,000 (Table 71, page 397), and their particle diameters from 10 to 100 μ . Lysed cultures of staphylococci contain about 1 γ per cent of phage. The phages seldom destroy all bacterial cells in a culture, and they must accumulate to definite threshold concentrations before bacterial lysis occurs. Hence, bacteriophage therapy in animals is most successful when the phage is introduced into circumscribed infected areas.

THE CHEMISTRY OF HEREDITY AND DEVELOPMENT

Virus and phage phenomena lead to a consideration of the relations of nucleoprotein metabolism to heredity. The *genes*, or fundamental units of inheritance, were shown by Mendel to be segregated and combined by chance, without loss of their identities. The genes have been associated with particular histological loci in the chromosomes, or giant nucleoprotein structures of dividing cell nuclei. These gene loci are arranged along the chromosomes in definite linear fashion. Natural mutations, x-ray treatment, and other processes produce deletions, duplications, inversions, and translocations of the loci, and qualitative transformations of individual genic characters. The genes which determine human blood group characteristics are examples of *allelomorphs* or alleles, that is, alternative genes, and they control the cellular synthesis of the blood group polysaccharide haptens. Virus nucleoproteins undergo similar chemical changes in their amino acid composition or structure, to form new virus strains within the cells of an infected host.

Alterations in chromosomal patterns accompany *mutation*, or changed inheritance of physiological and morphological characteristics. Mutations are most easily detected by changes in tissue colorations, shapes and sizes of organs, secondary sex characteristics or other functions, also by homologous organ transformations and altered developmental stages. A mutant gene is usually named, arbitrarily, from the most noticeable change induced in the organism; but many other interrelated or *pleiotropic* effects may result from each mutation. Numerous genes are therefore polyurgic (*i.e.*, cause several physiological effects). Only certain genic arrangements in the chromosomes allow continued stability of any particular organism in its reactions with the environment. The great majority of mutations give rise to freaks or monsters; more than 90 per cent of the known mutations are lethal to organisms at some stage of development. Since the isolated tissues of such animals can be grown satisfactorily, death from lethal genes is attributed to disturbed physiological correlation.

Rearrangements of gene loci and the nature of the particular substance at a given locus are both important in mutation. It would appear that certain genic nucleoproteins can assume the properties of others, whose

normal gene-string positions they may occupy. Also, a paired nucleoprotein structure located in a single chromosome, as the result of defective crossing over, acts differently from the same pair in normal apposition in the paired chromosomes. Undoubtedly, the positional relations of nucleoproteins in chromosomes are correlated with their chemical interactions. The chromosome is a definite system of nucleoproteins, whose dynamic activities determine the course of development; each species has a characteristic gene configuration. The genes represent regulatory or pace-making activities of enzymatic nucleoproteins, which control the rates of intricate and integrated chains of catalytic reactions.

Mutations can delay the liberation of certain morphogenetic hormones sometimes termed *evocators*, *inductors*, or *organizers*, and can thus prevent the normal concentrations of these substances necessary for particular phases of development. *Phenocopies*, or imitations of genic effects, can be produced by external agents, for example, the lack of metamorphosis in tadpoles deficient in iodine, and the formation of red eye pigments in insects by administered kynurenine. The latter substance is the essential component of the so-called *Drosophila* v^+ gene hormone, which induces vermilion eye color. Kynurenine formation from tryptophane is controlled by the *vermilion* gene. The kynurenine is oxidized to a pigment precursor (the cn^+ gene hormone) under the influence of the *cinnabar* gene. The v^+ hormone production is not dependent on genic effects alone; it can be accelerated by partial starvation. The number of mutant genes which affect a given biochemical synthesis gives an indication of the number of separate chemical reactions involved in the synthetic process.

Inductors are effective only during appropriate sensitive stages of development; subsequently, the pattern of the organism and its parts is no longer plastic. The best studied evocator is that which initiates the appearance of such primitive embryonic organs as the neural plate, notochord, and somites. During the development of totipotent or regulation type eggs, this substance is first liberated in the dorsal lip of the blastopore (roof of the archenteron). It diffuses slowly through neighboring cells and institutes chemical differentiations which determine the morphogenic possibilities of various cell areas. The developing organism thus becomes an irreversible chemical mosaic of unlike regions. This primary evocator is liberated from an inactive cellular precursor, by contact with dead cells of all descriptions, or by the action of such varied substances as methylene blue, glycogen, hydrocarbons, lipides, digitonin, protein, and acids. Secondary inductors, which induce other organ formations, appear at later stages of development. The sites of certain secondary evocators are as follows: nasal placode, in forebrain; eyecup, in midbrain; lens and cornea, in eyecup; ear vesicle, in hindbrain and head mesoderm; tympanic membrane, in annular tympanic cartilage; posterior hypophysis, in anterior notochord; anterior hypophysis, in brain and posterior hypophysis; teeth and mouth, in pharyngeal endoderm; gills, in branchial endoderm;

limb buds, in side plate mesoderm; heart and blood, in endoderm; pronephros, in mesoderm; mesonephros, in pronephric duct; and anus, in tail bud mesenchyme. Insects have a metamorphic gene hormone in the corpora allata, and moulting and pupation hormones in nerve tissue. The developing embryo is an orderly chemical and physical system whose organization is dependent on oxidation reactions. Under prolonged anaerobic conditions, the cells of the embryo tend to become relatively free and disorganized.

Certain mutations lead to degenerations through the formation of lytic substances, whose action resembles that of specific phages. Other mutations induce premature arrest of some developmental process, so that a partial embryonic condition persists in the adult. The *nana* gene of dwarf corn induces accelerated destruction of the plant growth hormone, auxin. The dwarf gene of mice influences the secretion of growth hormone by the anterior lobe of the pituitary. Such animals are restored to normal by injecting anterior pituitary growth hormone. In hereditary otocephaly of guinea pigs, the head organizer is abnormal, with resultant failure of ear, eye, and mouth inductors. Deficiency of eyecup evocator is a relatively frequent anomaly of chicks. The shaker-short mutation in mice produces deafness and labyrinth deficiency traceable to failure of brain expansion. In recessive whiteness, certain skin areas lack the enzymes which produce melanin; in dominant whiteness the enzymes are present, but an inhibitor is formed. Chondrodystrophy represents a disturbance of cartilage formation and perichondrial ossification. Human achondroplasia is an inherited dwarfism related to cartilaginous deficiency in the long bones. Lethal genes cause blastocyst degeneration in the yellow mouse, lack of mesoderm in the lethal mouse, notochord and limb bud deficiencies in the short tail mouse, limb bud failure in the creeper chick, and deficient erythropoiesis in the anemic mouse. The lethal genes are fatal only in doubled or homozygous combination. Haploid hybrids undergo abnormal development, and early death results. Experiments with parthenogenetic merogons (cells without nuclei) show that chromatin substances are necessary for development beyond the blastula stage. Gastrulation is impeded by gynogenesis (fertilization by injured sperm, with elimination of sperm nuclear substances from the developmental process) or by androgenesis (fertilization of an injured egg).

The cytoplasm is a mixture of substrates for the various genic actions; the chromosomal enzymes require precise substrates and coenzymic factors which vary in different cytoplasm. The results of any particular gene action, therefore, depend on the external environment, the cytoplasmic environment, and the interrelations with other genes (the internal genic environment). Cytoplasmic inheritance is minor; it may be due to the activity of cytoplasmic proteins, termed plasmagenes.

It has been calculated that genic nucleoprotein particles have molecular weights below 33,000,000, and are smaller than 20 by 125 μ . The inten-

sively studied *Drosophila* nuclei contain about 1,280 gene loci in the X chromosomes, and 13,100 in the autosomes. The skeleton of the chromosomes consists of such proteins as nucleohistones, protamines, and chromosomins. Ultraviolet absorption spectra show that the alternating light and dark staining transverse discs of *Drosophila* salivary chromosomes consist chiefly of complex proteins and nucleohistones, respectively. During the prophase of mitosis, deoxyribonucleic acid increases in the chromosomes, and the more complex proteins are replaced by histones; these changes are reversed during telophase. The chromosomes are the site of the nuclear thymonucleodepolymerase. The heterochromatin and nucleoli contain histone and ribonucleic acid. Sulfhydryl radicals of nuclei increase after fission and decrease at metaphase. The achromatic spindle is a gel; in most animal cells it arises outside the nucleus, and it encloses the nuclear sap as the nuclear membrane disappears and the chromosomes undergo longitudinal cleavage. Spindle fibers and astral rays are orientations of granules in the fibrillar elastic gel.

Between each cell division, the quantity of the genic nucleoproteins is doubled by a synthetic cellular activity which is similar to the autocatalytic synthesis of the viruses. The nucleoprotein strings are complex dynamic structures, formed by living cells under the directing influence of templates or organizing patterns. The chromosome system, like its constituent proteins and nucleoproteins, is an autocatalytic system which tends to establish point to point identity in the chromosome partners. The site of this attraction is the chromomeres, or individual granules along the chromosome. The nuclei of the somatic cells of most higher organisms normally contain two chromosomes of each type; when more than one pair are present, a condition of *polyploidy* exists. Colchicine and acenaphthene modify cell division by disturbing spindle formation and thus blocking the anaphase. Owing to the absence of a spindle, the split metaphase half-chromosomes do not separate. Since each chromosome divides before the nucleus is reconstructed, tetraploid nuclei are obtained (page 210). These drugs, therefore, cause polyploidy, enlargement of the cells and nuclei, multinucleation, increase in number of nucleoli, and abnormal mitosis. The physiological results of polyploidy are increased cell size, accelerated cell division, and general gigantism of the organism.

BIBLIOGRAPHY

CHEMISTRY

General

- BRADSTREET, R. B. Kjeldahl determination of organic nitrogen. *Chem. Rev.*, 27 : 331, 1940.
- JORDEN-LLOYD, D., and SHORE, A. Chemistry of the Proteins. Ed. 2. Philadelphia, Blakiston, 1938. (Methods.)

SAHYUN, M. Outline of the Amino Acids and Proteins. New York, Reinhold, 1944.

SCHMIDT, C. L. A. Chemistry of the Amino Acids and Proteins. Ed. 2. Springfield, Thomas, 1944. (Methods.)

Amino Acids

BLOCK, R. J., and BOLLING, D. The Amino Acid Composition of Proteins and Foods. Springfield, Thomas, 1945.

GILMAN, H. Organic Chemistry. Ed. 2. Vol. II. New York, Wiley, 1943.

Amino Acid Derivatives

FOX, S. W. Chemistry of the biologically important imidazoles. *Chem. Rev.*, 32 : 47, 1943.

GUOGENHEIM, M. Die Biogenen Amine. Ed. 3. New York, Nordemann, 1940.

Physical Chemistry of Proteins

ABRAMSON, H. A., *et al.* Electrophoresis of Proteins and the Chemistry of Cell Surfaces. New York, Reinhold, 1942.

COHN, E. J., and EDSALL, J. T. Proteins, Amino Acids and Peptides as Ions and Dipolar Ions. New York, Reinhold, 1942.

GREENBERG, D. M. The interaction between alkaline earth cations and proteins. *Adv. in Prot. Chem.*, 1 : 121, 1944.

LANGMUIR, I., and SCHAEFER, V. J. Properties and structures of protein monolayers. *Chem. Rev.*, 24 : 181, 1939.

LONGSWORTH, L. G. The study of proteins by electrophoresis. *Chem. Rev.*, 30 : 323, 1942.

NEURATH, H., *et al.* The chemistry of protein denaturation. *Chem. Rev.*, 34 : 157, 1944.

Structure and Molecular Weight of Proteins

ASTBURY, W. T. X-rays and the stoichiometry of the proteins. *Adv. in Enzymol.*, 3 : 63, 1943.

BULL, H. B. Protein structure. *Adv. in Enzymol.*, 1 : 1, 1941.

HUGGINS, M. L. The structure of fibrous proteins. *Chem. Rev.*, 32 : 195, 1943.

SVEDBERG, T., and PEDERSON, K. O. The Ultracentrifuge. Oxford, Clarendon Press, 1940.

SYNGE, R. L. M. Hydrolytic products of proteins and protein structure. *Chem. Rev.*, 32 : 135, 1943.

Individual Proteins

(See references to Chromoproteins, page 564.)

BAILEY, C. H. The Constituents of Wheat and Wheat Products. New York, Reinhold, 1944.

BAILEY, K. The proteins of skeletal muscle. *Adv. in Prot. Chem.*, 1 : 289, 1944.

CHARGAFF, E. Lipoproteins. *Adv. in Prot. Chem.*, 1 : 1, 1944.

- COHN, E. J. Properties and functions of the plasma proteins. *Chem. Rev.*, 28 : 395, 1941.
- JUKES, T. H., and KAY, H. D. Egg yolk proteins. *J. Nutrition*, 5 : 81, 1932.
- KOSSEL, A. The Protamines and Histones. New York, Longmans, Green, 1928.
- LEVENE, P. A. T. Hexosamines and Mucoproteins. New York, Longmans, Green, 1925.
- NORTHRUP, J. H. Crystalline Enzymes. New York, Columbia Univ. Press, 1939.
- OSBORNE, T. B. The Vegetable Proteins. Ed. 2. New York, Longmans, Green, 1924.
- SCHMITT, F. O. Structural proteins of cells and tissues. *Adv. in Prot. Chem.*, 1 : 25, 1944.
- SMITH, P. I. Glue and Gelatine. New York, Chem. Pub. Co., 1943.
- SPIEGEL-ADOLPH, M. Die Globuline. Dresden, T. Steinkopff, 1930.
- SUTERMEISTER, E. Casein and Its Industrial Applications. New York, Chemical Catalog Co., 1927.
- VICKERY, H. B. The proteins of plants. *Physiol. Rev.*, 25 : 347, 1945.

Hydrolysis of Proteins

- BERGMANN, M. A. Classification of proteolytic enzymes. *Adv. in Enzymol.*, 2 : 49, 1942.
- BERGMANN, M., and FRUTON, J. S. The specificity of proteinases. *Adv. in Enzymol.*, 1 : 63, 1941.
- BERGMANN, M., and FRUTON, J. S. The significance of coupled reactions for the enzymic hydrolysis and synthesis of proteins. *Ann. New York Acad. Sc.*, 45 : 409, 1944.
- JOHNSON, M. J., and BERGER, J. The enzymatic properties of peptidases. *Adv. in Enzymol.*, 2 : 69, 1942.
- MASCHMANN, E. Bacterial proteinases. *Ergebn. Enzymforsch.*, 9 : 155, 1943.

METABOLISM

General

- CHIBNALL, A. C. Protein Metabolism in the Plant. New Haven, Yale Univ. Press, 1939.
- JOHNSON, W. H. Nutrition in the protozoa. *Quart. Rev. Biol.*, 16 : 336, 1941.
- MITCHELL, H. H. The chemical and physiological relationship between vitamins and amino acids. *Vitamins and Hormones*, 1 : 157, 1943.
- RITTENBERG, D. The state of proteins in animals as revealed by the use of isotopes. *Cold Spring Harbor Symp. Quant. Biol.*, 9 : 283, 1941.
- SCHOENHEIMER, R. The Dynamic State of Body Constituents. Cambridge, Harvard Univ. Press, 1942.
- STEPHENSON, M. Bacterial Metabolism. Ed. 2. New York, Longmans, Green, 1939.
- WERKMAN, C. H., and WOOD, H. G. Metabolism of bacteria. *Botan. Rev.*, 8 : 1, 1942.

Transportation

- MYERS, V. C., and MUNTWYLER, E. Chemical changes in the blood and their clinical significance. *Physiol. Rev.*, 20 : 1, 1940.

Plasma Proteins; Synthesis; Autolysis

- ALCOCK, R. S. Synthesis of proteins *in vivo*. *Physiol. Rev.*, 16 : 1, 1936.
 ANDRUS, W. D., and LORD, J. W., JR. Physiology of plasma prothrombin. *Surgery*, 12 : 801, 1942.
 BRADLEY, H. C. Autolysis and atrophy. *Physiol. Rev.*, 18 : 173, 1938.
 MUDD, S., and THALHIMER, W. Blood Substitutes and Blood Transfusion. Springfield, Thomas, 1942.
 (Series of authors.) Chemical, clinical and immunological studies on products of human plasma fractionation. *J. Clin. Investigation*, 23 : 417-606, 1944.
 WHIPPLE, G. H., and MADDEN, S. C. Hemoglobin, plasma protein and cell protein. *Medicine*, 23 : 215, 1944.

Biological Value; Nitrogen Equilibrium

- BLOCK, R. J. The essential amino acid requirements of men. *Yale J. Biol. & Med.*, 15 : 723, 1943.
 CUTHBERTSON, D. P. Quality and quantity of protein in relation to human health and disease. *Nutrition Abstr. & Rev.*, 10 : 1, 1940.
 ELMAN, R. Maintenance of nitrogen balance by the intravenous administration of plasma proteins and protein hydrolyzates. *Physiol. Rev.*, 24 : 372, 1944.
 MARTIN, G. J., and THOMPSON, M. R. Intravenous alimentation with amino acids. *Medicine*, 22 : 73, 1943.

Deamination; Decarboxylation

- BLASCHKO, H. The amino acid decarboxylases of mammalian tissue. *Adv. in Enzymol.*, 5 : 67, 1945.
 HERBST, R. M. The transamination reaction. *Adv. in Enzymol.*, 4 : 75, 1944.

Melanin Pigments

- BLUM, H. F. The physiological effects of sunlight on man. *Physiol. Rev.*, 25 : 483, 1945.
 MARKOWITZ, M. Practical Survey of Chemistry and Metabolism of the Skin. Philadelphia, Blakiston, 1942.
 MEIROWSKY, E. Critical review of pigment research. *Brit. J. Dermat.*, 52 : 205, 1940.

Excretion

- VAN SLYKE, D. D. Renal mechanisms controlling composition of body fluids. *Chem. Rev.*, 26 : 105, 1940.

Sulfur Metabolism

- LEWIS, H. B. The significance of sulfur-containing amino acids in metabolism. *Harvey Lect.*, 36 : 159, 1940-41.
 SMYTHE, C. V. Some enzyme reactions on sulfur compounds. *Adv. in Enzymol.*, 5 : 237, 1945.

Creatine and Creatinine

BEARD, H. H. *Creatine and Creatinine Metabolism*. New York, Chemical Pub. Co., 1943.

CHALLENGER, F. Biological methylation. *Chem. Rev.*, 36 : 315, 1945.

VAGUE, J., and DUNAN, J. *La créatine*. Paris, Masson, 1939.

PATHOLOGY

Nephritis; Nephrosis; Hypertension

ALTNOW, H. O., *et al.* Renal amyloidosis. *Arch. Int. Med.*, 63 : 249, 1939.

FISHBERG, A. M. *Hypertension and Nephritis*. Ed. 4. Philadelphia, Lea and Febiger, 1939.

FORSTER, R. E. Thiocyanates in the treatment of arterial hypertension. *Am. J. M. Sc.*, 206 : 668, 1943.

GOLDRING, W., and CHASIS, H. *Hypertension and Hypertensive Disease*. New York, Commonwealth Fund, 1944.

HARRISON, T. R., and MASON, M. F. The pathogenesis of the uremic syndrome. *Medicine*, 16 : 1, 1937.

HERRIN, R. C. Factors affecting tests of kidney function. *Physiol. Rev.*, 21 : 529, 1941.

LEWIS, H. A., and GOLDBLATT, H. The humoral mechanism of hypertension. *Bull. New York Acad. Med.*, 18 : 459, 1942.

PAGE, I. H., and CORCORAN, A.C. *Arterial Hypertension, Its Diagnosis and Treatment*. Chicago, Year Book Pub., 1945.

WEISS, S., and PARKER, F. Pyelonephritis. *Medicine*, 18 : 221, 1939.

Toxemias of Pregnancy

DIECKMANN, W. J. *The Toxemias of Pregnancy*. St. Louis, Mosby, 1941.

Anomalies of Protein Metabolism

ANDREWS, J. C., and RANDALL, A. Sulfur metabolism in cystinuria. *J. Clin. Investigation*, 14 : 517, 1935.

BLACKBERG, S. N., and WANGER, J. O. Melanuria. *J. A. M. A.*, 100 : 334, 1933.

HOWARD, C. P., and MILLS, E. S. Ochronosis. In *Oxford Medicine*. Vol. 4, pt. 1, p. 223. London, Oxford Univ. Press, 1936.

JERVE, G. A. Phenylpyruvica oligophrenia. *Arch. Neurol. & Psychiat.*, 38 : 944, 1937.

PEACOCK, S. C., and KNOWLTON, K. Alkaptonuria. *Am. J. Dis. Child.*, 56 : 100, 1938.

Muscular Diseases

(See references to Cholinergic Diseases, page 260.)

ARING, C. D., and COBB, S. Muscular atrophies and allied disorders. *Medicine*, 14 : 77, 1935.

PAPPENHEIMER, A. M. Muscle disorders associated with vitamin E deficiency. *Physiol. Rev.*, 23 : 37, 1943.

- WANG, E. Clinical and experimental investigations on creatine metabolism. *Acta Med. Scandinav.*, Suppl. 105, 1939.
- WILSON, S. A. K. *Neurology*. Vol. II. Baltimore, Williams and Wilkins, 1940.

Miscellaneous

(See references to Cerebrospinal Fluid, page 349.)

- BLACK, D. A. K. Azotemia in gastro-duodenal hemorrhage. *Quart. J. Med.*, 11 : 77, 1942.
- BLALOCK, A. Peripheral circulatory failure. *Am. Heart J.*, 23 : 147, 1942.
- HARKINS, H. N. Recent advances in the study and management of traumatic shock. *Surgery*, 9 : 231, 447, 607, 1941.
- HARKINS, H. N. The Treatment of Burns. Springfield, Thomas, 1942.
- HARKINS, H. N. The present status of the problem of thermal burns. *Physiol. Rev.*, 25 : 531, 1945.
- HOUSSAY, B. A. Phenolemia and indoxylemia. *Am. J. M. Sc.*, 192 : 615, 1936.
- JANEWAY, C. A. Plasma proteins in clinical medicine and surgery. *New England J. Med.*, 229 : 751, 779, 1943.
- JEGHERS, H., and BAKST, H. J. Syndrome of extrarenal azotemia. *Ann. Int. Med.*, 11 : 1861, 1938.
- LUND, C. C., and LEVENSON, S. M. Protein in surgery. *J. A. M. A.*, 128 : 95, 1945.
- MEYLER, L. Bence-Jones' proteinuria. *Arch. Int. Med.*, 57 : 708, 1936.
- MOON, V. H. Shock: Its Dynamics, Occurrence and Management. Philadelphia, Lea and Febiger, 1942.
- SCUDDER, J. Shock; Blood Studies as a Guide to Therapy. Philadelphia, Lippincott, 1940.
- STARE, F. J., and THORN, G. W. Protein nutrition in problems of medical interest. *J. A. M. A.*, 127 : 1120, 1945.
- WIGGERS, C. J. The present status of the shock problem. *Physiol. Rev.*, 22 : 74, 1942.

IMMUNITY AND HEREDITY

Chemistry of Immunity

- BOYD, W. C. Fundamentals of Immunology. New York, Interscience Pub. 1943.
- BURNET, F. M. Production of Antibodies. Melbourne, Macmillan, 1941.
- CANNON, P. R. The functional significance of specific agglutinins and precipitins. *Physiol. Rev.*, 20 : 89, 1940.
- DURAN-REYNOLDS, F. Tissue permeability and the spreading factor in infection. *Bact. Rev.*, 6 : 197, 1942.
- EATON, M. D. Recent chemical investigations of bacterial toxins. *Bact. Rev.*, 2 : 3, 1938.
- ESSEX, H. E. Certain animal venoms and their physiologic action. *Physiol. Rev.*, 25 : 148, 1945.
- KABAT, E. A. Immunochemistry of the proteins. *J. Immunol.*, 47 : 513, 1943.
- KAHN, R. L. Tissue Immunity. Springfield, Thomas, 1936.
- LANDSTEINER, K. The Specificity of Serological Reactions. Ed. 2. Cambridge, Harvard Univ. Press, 1945.

- MENKIN, V. The role of inflammation in immunity. *Physiol. Rev.*, 18 : 366, 1938.
- PAULING, L., *et al.* The nature of the forces between antigen and antibody. *Physiol. Rev.*, 23 : 203, 1943.
- PILLEMER, L. Recent advances in the chemistry of complement. *Chem. Rev.*, 33 : 1, 1943.
- SEIBERT, F. B. The chemistry of tuberculin. *Chem. Rev.*, 34 : 107, 1944.
- SHWARTZMAN, G. Phenomenon of Local Tissue Reactivity. New York, Hoeber, 1937.
- THOMPSON, R. Lysozyme. *Arch. Path.*, 30 : 1096, 1940.
- TREFFERS, H. P. Contributions of immunology to the study of proteins. *Adv. in Prot. Chem.*, 1 : 69, 1944.

Leukocytes; Phagocytosis

- DOWNEY, H. Handbook of Hematology. New York, Hoeber, 1938. (4 vol.)
- FORKNER, C. E. Leukemia and Allied Disorders. New York, Macmillan, 1938.
- MUDD, S., *et al.* Phagocytosis. *Physiol. Rev.*, 14 : 210, 1934.

Immunity in Disease

- CECIL, R. L. Nonspecific protein therapy. *J. A. M. A.*, 105 : 1846, 1935.
- CULBERTSON, J. T. Immunity Against Animal Parasites. New York, Columbia Univ. Press, 1941.
- GAY, F. P., *et al.* Agents of Disease and Host Resistance. Springfield, Thomas, 1935.
- HOLMES, E. The effect of toxemia on metabolism. *Physiol. Rev.*, 19 : 439, 1939.
- KELLAWAY, C. H. Snake venoms. *Bull. Johns Hopkins Hosp.*, 60 : 1, 1937.
- KOLMER, J. A., and TUFT, L. Clinical Immunology, Biotherapy and Chemotherapy. Philadelphia, Saunders, 1942.
- WELLS, H. G., and LONG, E. R. The Chemistry of Tuberculosis. Ed. 2. Baltimore, Williams and Wilkins, 1932.
- ZINSSER, H., *et al.* Immunity. Ed. 5. New York, Macmillan, 1940.

Chemotherapy

- DUBOS, R. J. Utilization of selective microbial agents in the study of biological problems. *Harvey Lect.*, 35 : 223, 1939-40.
- HENRY, R. J. The Mode of Action of Sulfonamides. New York, Josiah Macy Jr. Foundation, 1944.
- HERRELL, W. E. Penicillin and Other Antibiotic Agents. Philadelphia, Saunders, 1945.
- HOTCHKISS, R. D. Gramicidin, tyrocidine and tyrothricin. *Adv. in Enzymol.*, 4 : 153, 1944.
- KOLMER, J. A. Penicillin Therapy. New York, Appleton-Century, 1945.
- POTH, E. J. The sulfonamides as therapeutic agents in intestinal antisepsis. *Internat. Abstr. Surg.*, 78 : 373, 1944.
- SPINK, W. W. Sulfanilamide and Related Compounds in General Practice. Ed. 2. Chicago, Year Book Pub., 1942.
- WAXSMAN, S. A. Microbial Antagonism and Antibiotic Substances. New York, Commonwealth Fund, 1945.

Hypersensitivity; Allergy

- COCA, A. F. Familial Nonreaginic Food Allergy. Ed. 2. Springfield, Thomas, 1945.
- DRAGSTEDT, C. A. Anaphylaxis. *Physiol. Rev.*, 21 : 563, 1941.
- FARMER, L. Histamine in anaphylaxis and allergy. *Bull. New York Acad. Med.*, 16 : 618, 1940.
- NEWELL, J. M. The allergens in pollens. *J. Allergy*, 13 : 177, 1942.
- RATNER, B. Allergy, Anaphylaxis and Immunotherapy. Baltimore, Williams and Wilkins, 1943.
- RICH, A. R. The significance of hypersensitivity in infections. *Physiol. Rev.*, 21 : 70, 1941.
- SULZBERGER, M. B. Dermatologic Allergy. Springfield, Thomas, 1940.
- URBACH, E. Allergy. New York, Grune and Stratton, 1943.

Viruses

- BAWDEN, F. C. Plant Viruses and Virus Diseases. Waltham, Chronica Botanica, 1943.
- PIRIE, N. W. Physical and chemical properties of plant viruses. *Adv. in Enzymol.*, 5 : 1, 1945.
- RIVERS, T. M., *et al.* Virus Diseases. Ithaca, Cornell Univ. Press, 1943.
- SEIFFERT, G. Virus Diseases in Man, Animal and Plant. New York, Philosophical Library, 1944.
- STANLEY, W. M. The architecture of viruses. *Physiol. Rev.*, 19 : 524, 1939.
- VAN ROOYEN, C. E., and RHODES, A. J. Virus Diseases of Man. London, Oxford Univ. Press, 1940.

Bacteriophages

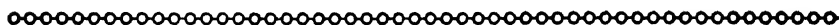
- DELBRUCK, M. Bacterial viruses. *Adv. in Enzymol.*, 2 : 1, 1942.

Chemistry of Heredity and Development

- BLOOM, W. Cellular differentiation and tissue culture. *Physiol. Rev.*, 17 : 589, 1937.
- GLASS, B. Genes and the Man. New York, Columbia Univ. Press, 1943.
- GOLDSCHMIDT, R. B. Physiological Genetics. New York, McGraw-Hill, 1938.
- GULICK, A. The chemical formulation of gene structure and action. *Adv. in Enzymol.*, 4 : 1, 1944.
- LOEB, L. The Biological Basis of Individuality. Springfield, Thomas, 1945.
- MIRSKY, A. E. Chromosomes and nucleoproteins. *Adv. in Enzymol.*, 3 : 1, 1943.
- NEDHAM, J. Biochemistry and Morphogenesis. London, Cambridge Univ. Press, 1942.
- ROBERTS, J. A. F. An Introduction to Medical Genetics. London, Oxford Univ. Press, 1940.
- (Series of authors.) Symposium on polyploidy. *Biol. Symposia*, 4 : 93, 1941.
- SMITH, C. A. Physiology of the Newborn Infant. Springfield, Thomas, 1945.
- SNYDER, L. H. Medical Genetics. Durham, Duke Univ. Press, 1941.
- WADDINGTON, C. H. Organizers and Genes. London, Cambridge Press, 1940.
- WHITE, M. J. D. The Chromosomes. Ed. 2. London, Methuen, 1942.
- WINDLE, W. F. Physiology of the Fetus. Philadelphia, Saunders, 1940.
- WRIGHT, S. The physiology of the gene. *Physiol. Rev.*, 21 : 487, 1941.

CHAPTER VII

PROSTHETIC RADICALS OF NUCLEOPROTEINS AND CHROMOPROTEINS



CHEMISTRY OF NUCLEIC ACIDS

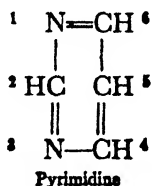
"Science is an exploration of the unknown; and it need not surprise us that prediction as to the outcome of few other human adventures is as hazardous as that concerning the direction which the future progress of any science will take." — MORRIS R. COHEN

TYPES OF NUCLEIC ACIDS

The nucleic acid molecule contains phosphoric acid, carbohydrate, and four nitrogenous bases (two purines and two pyrimidines). The carbohydrate of so-called "plant" nucleic acid is *d*-ribose, and that of "animal" nucleic acid is *d*-2-desoxyribose. Since each of these nucleic acids occurs in animals, plants, bacteria, and viruses, the terms *ribonucleic* and *desoxyribonucleic* are more appropriate. The purines, adenine and guanine, and the pyrimidine, cytosine, are found in both acids. The second pyrimidine unit of ribonucleic acid is uracil; in ordinary desoxyribonucleic acid it is thymine, and in the desoxyribonucleic acid of certain bacteria it is methylcytosine. Hydrolysis of the nucleic acids, and subsequent oxidation of the nitrogenous units, produces the derivatives classified in Table 84.

PYRIMIDINES

These nitrogenous substances are derivatives of the heterocycle, pyrimidine or 1,3-diazine.



The structures and distribution of the pyrimidines of biological interest are given in Table 85. Uracil and thymine are oxidative deamina-

TABLE 84

CLASSIFICATION OF NUCLEIC ACIDS
AND THEIR DERIVATIVES

<i>Pyrimidines</i> . . .	Amino and oxy derivatives of pyrimidine. <i>Examples:</i> cytosine, methylcytosine, thymine, and uracil of nucleic acids; alloxan, barbituric acid, divicine, orotic acid.
<i>Purines</i> . . .	Amino and oxy derivatives of purine. <i>Examples:</i> adenine and guanine of nucleic acids; oxidized metabolites: hypoxanthine, xanthine, uric acid; methylpurines: caffeine, theobromine, theophyllin; pterin pigments: chrysopterin, erythropterin, guanopterin, leucopterin, mesopterin, xanthopterin.
<i>Nucleosides</i> . . .	Purine or pyrimidine N-glycosides of ribose, desoxyribose, glucose, or thiomethylpentose. <i>Examples:</i> <i>d</i> -ribosides: adenosine, cytidine, guanosine, inosine, uric acid riboside, uridine, xanthosine; <i>d</i> -desoxyribosides: desoxyadenosine, desoxycytidine, desoxyguanosine, thymidine; <i>d</i> -glucoside: vicine; thiomethylpentoside: adenine thiomethylpentoside.
<i>Nucleotides</i> . . .	Phosphoric acid esters of nucleosides.
<i>Mononucleotides</i> .	Contain one molecule each of phosphoric acid, sugar, and purine or pyrimidine base. <i>Examples:</i> ribonucleotides: cytidylic acid, cytoflavin, ¹ guanylic acid, muscle adenylic acid, muscle inosinic acid, uridylic acid, xanthylic acid, yeast adenylic acid, yeast inosinic acid.
<i>Dinucleotides</i> . .	Compounds of two mononucleotides. <i>Examples:</i> pyridine nucleotides: cozymase I, cozymase II; flavin-adenine dinucleotide ¹ (prosthetic radical of diaphorase).
<i>Tetranucleotides</i> .	Compounds of four mononucleotides, esterified through carbohydrate and phosphoric acid radicals. <i>Examples:</i> depolymerized molecules of the nucleic acids of α -nucleoproteins.
<i>Polynucleotides or native nucleic acids</i>	High molecular polymers of tetra-, penta-, or hexa-nucleotides. <i>Examples:</i> the ribo- or "plant" nucleic acids: nucleic acid of pancreatic β -nucleoprotein, triticonucleic acid of wheat, yeast nucleic acid; desoxyribo- or "animal" nucleic acids: thymonucleic acid; tuberculinic acid.

¹ The flavin nucleotides contain the sugar alcohol, *d*-ribitol, in place of *d*-ribose.

tion products of cytosine and methylcytosine, respectively. Synthetic barbituric acid derivatives are important anesthetics and hypnotics. Alloxan is an oxidation product of uric acid. In acid solution, oxidation leads to the formation of keto or oxy radicals at the pyrimidine carbon atoms 5 and 6; but in alkaline solution, oxidation ruptures the ring and produces urea. One of the rings of thiamin (vitamin B₁) is 2,5-dimethyl-6-aminopyrimidine; riboflavin (vitamin B₂) also contains a pyrimidine ring.

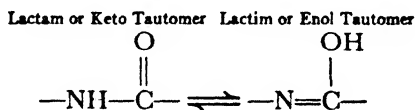
TABLE 85

PYRIMIDINES

BIOLOGICAL NAME	CHEMICAL NAME	NATURAL SOURCE
Cytosine	2-Oxy-6-aminopyrimidine	Ribo- and desoxyribo-nucleic acids
Uracil	2,6-Dioxypyrimidine	Ribonucleic acids
Methylcytosine	2-Oxy-6-amino-5-methylpyrimidine	Bacterial desoxyribonucleic acids
Thymine	2,6-Dioxy-5-methylpyrimidine	Desoxyribonucleic acids
Divicine	2,5-Diamino-4,6-dioxypyrimidine	Vicine
Orotic acid	Uracil-4-carboxylic acid	Free in milk
Barbituric acid	2,4,6-Trioxypyrimidine	
Alloxan	2,4,5,6-Tetraoxypyrimidine	

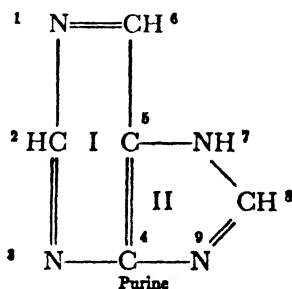
Sulfadiazine and sulfamerazine are pyrimidine derivatives of sulfanilamide (page 477).

Pyrimidines are precipitated by silver nitrate only in neutral or alkaline solution, whereas the purines can be precipitated by this reagent in acid or alkaline solution. The oxy derivatives of pyrimidines and purines are ampholytes. (See Table 1, page 4, for dissociation constants.) The oxy-pyrimidines and oxypurines exist in tautomeric forms, which are produced by the migration of hydrogen atoms. The tautomeric change may be represented as follows:



PURINES

These substances are derivatives of the nitrogenous heterocycle, purine.



Ring I is the pyrimidine heterocycle; ring II is the glyoxaline or imidazole nucleus, which is also present in the amino acid, histidine. The purines of

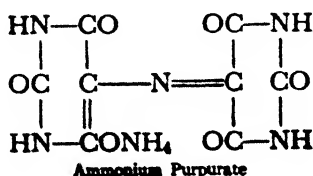
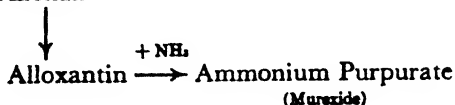
biological interest are listed in Table 86. Adenine and guanine are deaminated by adenase and guanase to form hypoxanthine and xanthine, respectively. Hypoxanthine, xanthine, and uric acid represent progressive stages in the oxidation of purines. These oxypurines are ampholytes (Table 1, page 4), and they exist in lactam and lactim forms. Small traces of methyl purines (1-, 3-, and 7-monomethyl, and 1,7-dimethyl derivatives) are excreted in the urine, as metabolites of the dietary methylpurines.

TABLE 86
PURINES

BIOLOGICAL NAME	CHEMICAL NAME	NATURAL SOURCE
Adenine	6-Aminopurine	Tea leaves, nucleic acids
Guanine	2-Amino-6-oxypurine	Tissues, nucleic acids
Hypoxanthine	6-Oxypurine	Tissues, inosinic acid
Xanthine	2,6-Dioxypurine	Tissues
Uric acid	2,6,8-Trioxypurine	Tissues, uric acid riboside
Theophylline	1,3-Dimethylxanthine	Tea leaves
Paraxanthine	1,7-Dimethylxanthine	Tissues
Theobromine	3,7-Dimethylxanthine	Chocolate, cocoa seeds, cola nuts
Caffeine	1,3,7-Trimethylxanthine	Coffee beans and leaves, cola nuts, cocoa seeds, and certain tea leaves
Tetramethyluric acid	1,3,7,9-Tetramethyluric acid	Tea leaves

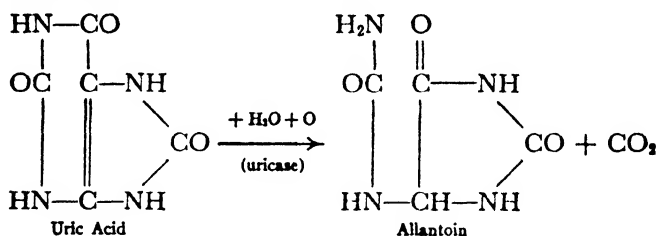
Adenine and guanine are precipitated by picric acid; and, together with hypoxanthine, cytosine, and methylcytosine, they form insoluble phosphotungstates. Caffeine, guanine, theophylline, uric acid, and xanthine give the *murexide test* on evaporation with a few drops of concentrated nitric acid. In this test, guanine and xanthine give yellow colors, while the other purines generate red colorations. Addition of ammonium or potassium hydroxide changes the colors to purple. The oxidative reactions which occur in the murexide test may be illustrated as follows:

Uric Acid \longrightarrow Dialuric Acid + Alloxan

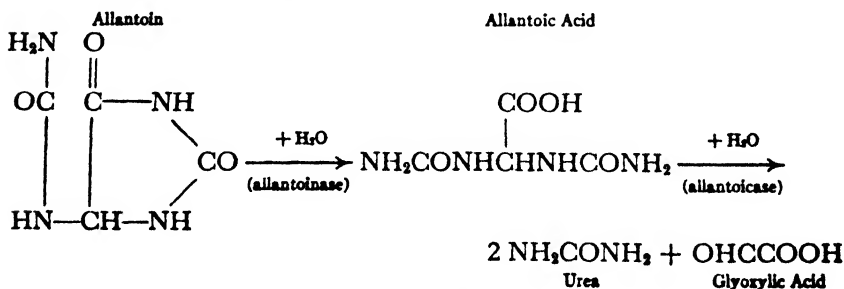


Uric acid is a weak dibasic acid, which forms both neutral and acid salts; the ionizable hydrogens of the lactim form are at carbons 2 and 8. At body temperature, water dissolves only 0.0065 per cent of uric acid, 0.141 per cent of sodium acid urate, and 0.054 per cent of ammonium acid urate. Lithium acid urate is more soluble (1.67 per cent at 18° C.). Uric acid can be determined colorimetrically, in protein-free filtrates of biological fluids, by Folin's phosphotungstic acid reagent or by Benedict's arsenophosphotungstic acid reagent, both of which are reduced to blue colored compounds. Since these reagents are not specific for uric acid, accurate determination requires the use of uricase preparations. In alkaline solution, uric acid has sufficient reducing activity to give false sugar tests with copper reagents, but the normal urinary concentrations do not interfere with Benedict's qualitative reagent or with Sumner's dinitrosalicylate reagent.

Complete hydrolysis and oxidation of purines, in acid solution, causes rupture of the imidazole ring with the formation of urea and alloxan from uric acid, and urea and 6-aminopyrimidine from adenine. Oxidation in alkaline solution ruptures the pyrimidine ring to form imidazole derivatives. This reaction is also catalyzed by the enzyme, uricase.



Allantoin is found in plants, and in the urine and tissue fluids of most mammals; it can be hydrolyzed to urea and glyoxylic acid by the enzymes, allantoinase and allantoinase.



NUCLEOSIDES

The nucleosides are N-glycosides of purines or pyrimidines, in which the reducing carbon of a sugar is united to pyrimidine nitrogen atom 3, or

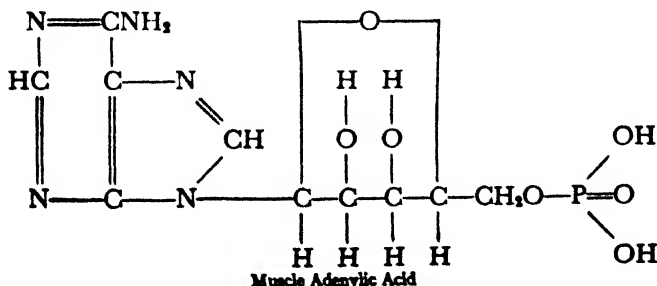
purine nitrogen atom 9. Nucleosides may be classified as ribosides, desoxyribosides, glucosides, or methylthiopentosides (Table 87). The ribosides and desoxyribosides are important intermediate hydrolytic products of nucleic acids; the sugar units of these nucleosides are in the furanoid form. Desoxyribose is destroyed by acid hydrolysis. Desoxyribosides not listed in the table are named from the corresponding ribosides; for example, desoxyadenosine. The pyrimidine ribosides and desoxyribosides are not found free in nature. They are more resistant to acid hydrolysis than are the purine nucleosides. Nucleoside linkages are not hydrolyzed by alkali. The isoelectric points of the nucleosides are in a pH range of 5.4 to 8.2. (See Table 1, page 4, for dissociation constants.)

TABLE 87
NUCLEOSIDES

BIOLOGICAL NAME	CHEMICAL NAME	NATURAL SOURCE
Adenosine	9-Adenine- <i>d</i> -riboside	Ribonucleic acids
Guanosine	9-Guanine- <i>d</i> -riboside	Free in tissues
Inosine	9-Hypoxanthine- <i>d</i> -riboside	Inosinic acid
Xanthosine	9-Xanthine- <i>d</i> -riboside	
Uric acid riboside	9-Uric acid- <i>d</i> -riboside	Free in liver, erythrocytes
Cytidine	3-Cytosine- <i>d</i> -riboside	Ribonucleic acids
Uridine	3-Uracil- <i>d</i> -riboside	Ribonucleic acids
Thymidine	3-Thymine- <i>d</i> -desoxyriboside	Desoxyribonucleic acids
Vicine	3-Divicine- <i>d</i> -glucoside	Free in plant tissues
Adenine methylthiopentoside		Yeast

NUCLEOTIDES

Esterification of the sugar radical of a nucleoside with phosphoric acid produces a *mononucleotide*. These esters may be classified as 3- and 5-phosphoribosides, according to the position of the phosphoric acid radical (Table 88). With the exception of guanylic acid, the 3-phosphoribosides are found only as units of nucleic acids, while the 5-phosphoribosides exist free in animal tissues. Yeast and muscle adenylc acids have the following structures:



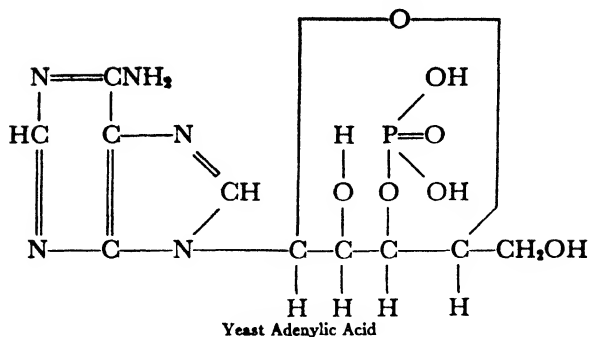


TABLE 88
NUCLEOTIDES

A. MONONUCLEOTIDES		
BIOLOGICAL NAME	CHEMICAL NAME	NATURAL SOURCE ¹
Muscle adenylic acid	9-Adenine-5-phosphoriboside	Free in tissues; cozymase I; flavin-adenine dinucleotide
Yeast adenylic acid	9-Adenine-3-phosphoriboside	Ribonucleic acids
Guanylic acid	9-Guanine-3-phosphoriboside	Ribonucleic acid; free in tissues
Muscle inosinic acid	9-Hypoxanthine-5-phosphoriboside	Free in tissues
Yeast inosinic acid	9-Hypoxanthine-3-phosphoriboside	
Xanthylic acid	9-Xanthine-3-phosphoriboside	
Cytidylic acid	3-Cytosine-3-phosphoriboside	Ribonucleic acids
Uridylic acid	3-Uracil-3-phosphoriboside	Ribonucleic acids
Adenosine pyrophosphoric acid	9-Adenine-5-diphosphoriboside	Free in tissues; cozymase II
Adenosine triphosphoric acid	9-Adenine-5-triphosphoriboside	Free in tissues
Cytoflavin (flavin nucleotide)	Riboflavin-5'-phosphoric acid	Free in tissues
Pyridine nucleotide	1-Nicotinamide-5-phosphoriboside	Cozymases
B. DINUCLEOTIDES		
BIOLOGICAL NAME	MONONUCLEOTIDE COMPONENTS	NATURAL SOURCE ¹
Cozymase I	Adenylic acid, pyridine nucleotide	Free in tissues
Cozymase II	Adenosine pyrophosphoric acid, pyridine nucleotide	Free in tissues
Flavin-adenine dinucleotide	Adenylic acid, cytoflavin	Free in tissues

¹ The designation "free" indicates that the nucleotide is not present as a nucleic acid. All nucleotides combine readily with proteins, as exemplified by the formation of the "yellow enzymes" (page 103).

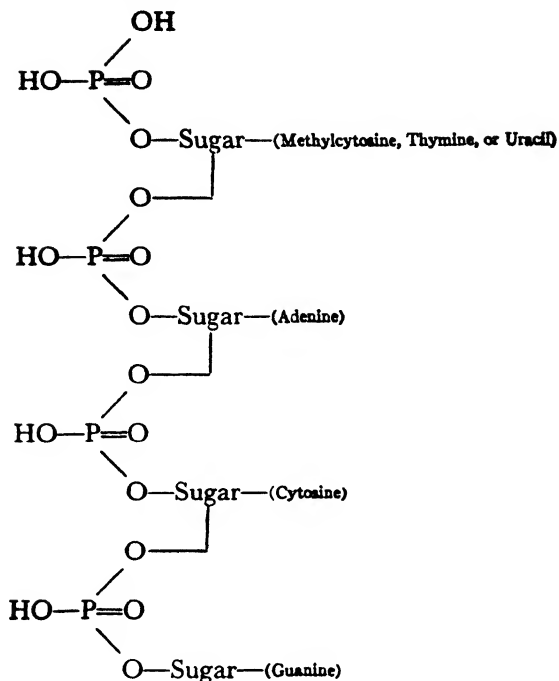
They are converted to the corresponding inosinic acids by specific adenylic acid deaminases (adenylases). Muscle adenylic acid unites readily *in vivo* with two molecules of phosphoric acid to form adenosine triphosphate, the ester of adenosine and triphosphoric acid. The significance of this phosphorylation in carbohydrate metabolism has been discussed on page 318. A pyrophosphoric or diphosphoric ester of adenosine, which represents a partial hydrolytic product of adenosine triphosphate, is found in heart muscle. Cytoflavin and nicotinamide phosphoriboside (pages 103 and 101) may be classified as flavin and pyridine nucleotides, respectively, although the flavin nucleotides contain the sugar alcohol, *d*-ribitol, in place of a sugar unit. The *dinucleotide* cozymases and flavin-adenine dinucleotide have been discussed on pages 101 and 103. They are compounds of adenylic acid with pyridine nucleotide and cytoflavin, respectively. Their mononucleotide units are united through the phosphoric acid radicals.

Nucleotides are acidic substances; their isoelectric points are in a pH range of 1.5 to 4.5. (See Table 1, page 4, for dissociation constants.) Nucleotides tend to form insoluble salts with heavy metal cations. Pyrimidine nucleotides are more difficultly hydrolyzed than purine nucleotides, and the 5-phosphoribosides less readily than the 3-phosphoribosides. Acid hydrolysis tends to cleave the nucleoside linkage, with liberation of the purine or pyrimidine, whereas alkali hydrolysis liberates the phosphoric acid. Nucleotides are hydrolyzed by non-specific phosphatases (page 597). Many tissues contain a specific 5-nucleotidase, which hydrolyzes only 5-phosphoribosides, and a pyrophosphatase, which hydrolyzes the pyrophosphate nucleotides to ordinary nucleotides and phosphoric acid.

POLYNUCLEOTIDES AND NUCLEIC ACIDS

The nucleic acids are high molecular polymers of nucleotides. The molecular weight of thymonucleic acid, as determined by the ultracentrifuge, ranges from 500,000 to 1,200,000, indicating the presence of 1,600 to 3,800 mononucleotide units. Yeast nucleic acid has a molecular weight of 17,000. In the large nucleic acid molecules, there is usually a repetition of a fundamental tetranucleotide pattern; two purine and two pyrimidine units are generally present in equimolecular proportions. However, the smallest chemical unit of the ribonucleic acid from pancreatic β -nucleoprotein is, apparently, a pentanucleotide or a hexanucleotide, which contains the four customary nucleotides and additional guanylic acid. Tetranucleotide units of nucleic acids may be represented as shown on the next page. The mononucleotide units are joined at carbon 2 of the sugar in ribonucleic acid, and at carbon 3 or carbon 5 in deoxyribonucleic acid.

Native nucleic acids consist of long chains of nucleotides joined in this fashion. Molecules of thymonucleic acid are fibrous; their length is three



hundred times their width. X-ray studies indicate a repeated intramolecular pattern of approximately seventeen mononucleotide units. The distance between adjacent mononucleotide units of nucleic acids is practically identical with that between adjacent amino acid side chains of fully extended fibrous proteins (3.34 Å). This relationship facilitates ready combination of nucleic acid and protein through polar linkages. Ordinary tissue nucleoproteins contain from 40 to 70 per cent of nucleic acid. Since tobacco mosaic virus contains only 5.8 per cent nucleic acid, it is estimated that its mononucleotide units combine with each fifty-fourth amino acid unit of the protein moiety. Tobacco mosaic virus ribonucleic acid has a molecular weight of approximately 300,000. Equine encephalomyelitis virus contains 4.4 per cent, swine influenza virus 5 per cent, vaccinia virus 5.6 per cent, rabbit papilloma virus 6.8 per cent, Rous sarcoma virus 10 per cent, tobacco necrosis and tomato bushy stunt viruses 15 per cent, and tobacco ring spot virus 40 per cent of nucleic acid.

The older classification of desoxyribonucleic and ribonucleic acids as "animal" and "plant" nucleic acids does not indicate their biological distribution. Both types are found in animals, plants, bacteria, and viruses. Thus, the β -nucleoproteins of mammalian pancreas and of chick embryo contain ribonucleic acids; these acids are found largely in the cytoplasm and nucleoli of plant and animal cells, and in plant viruses, and Rous

sarcoma virus and equine encephalomyelitis virus. The basophilic property of cytoplasm is attributable chiefly to its ribonucleic acids. Desoxyribonucleic acids are present in the nuclei of plant and animal tissues, in the nuclear-like material of bacteria, in influenza and rabbit papilloma viruses, and in the inclusion bodies associated with vaccinia and psittacosis viruses. In Feulgen's nuclear reaction, desoxyribonucleic acids give a blue-violet coloration when the tissue is treated with N hydrochloric acid solution and fuchsin-sulfite reagent. Both types of nucleic acid, as well as free purines and pyrimidines, absorb ultraviolet light at 260 $m\mu$.

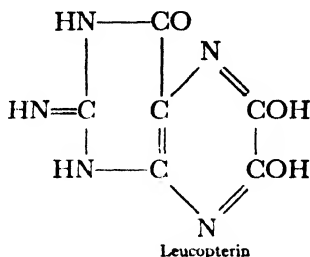
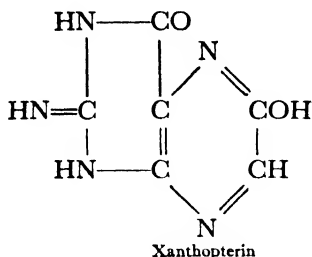
Nucleic acids are present in largest concentration in heavily nucleated tissues; they account for 60 per cent of the total solids in defatted sperm, 15 to 20 per cent in certain bacteria, 13 per cent in the thymus gland, 10 per cent in the pancreas, and 7 per cent in yeast. These acids are colloids, with isoelectric points below pH 2. They dissolve readily in alkali, and are flocculated by acids. The nucleic acids are difficultly soluble in water, but imbibe it readily and tend to produce slimy gels. Sodium thymonucleate imbibes as much as 4,000 times its weight of water. Nucleic acids are classified as α (high molecular) and β (low molecular) acids, depending on whether or not they form gels; this nomenclature has no relation to the older classification of α - or desoxyribo-nucleoproteins, and β - or ribonucleoproteins of animal tissues. α -Nucleic acids can be depolymerized to β -acids by heating in alkaline solution or by the action of the enzyme, nucleogelase. Ribonuclease and thymonucleodepolymerase are specific enzymes for the depolymerization of ribonucleic and desoxyribonucleic acids, respectively.

Owing to their relatively high dissociation (Table 1, page 4), the nucleic acids unite readily with inorganic cations and with proteins. Heavy metal and alkaline earth nucleates are insoluble, as are the nucleic acid salts of phosphotungstic, picric, and tannic acids. In slightly acid solution, the nucleic acids form insoluble compounds with proteins; these are *nucleins*. The nucleic acids are readily separated from their salt linkage with protein by the addition of alkali. Hydrolysis with dilute ammonia frees the mononucleotide units, while greater concentrations of alkali tend to produce phosphate and nucleosides. Phosphoproteins differ from nucleic acids in that alkali more readily frees the phosphoric acid of the phosphoproteins. Acids hydrolyze nucleic acids completely to purines, pyrimidines, sugar, and phosphoric acid.

PURINE PIGMENTS

The *pterins* are white, yellow, or red pigments found in small quantities in many living organisms; especially large concentrations are present in the integument of certain insects and snakes and in the wings of birds and butterflies. Xanthopterin is a yellow pigment found in mammalian urine,

and in the integument of wasps and hornets; it has antianemic properties. Leucopterin is a white substance in butterfly wings. Other pterins are listed in Table 84, page 507. The pterins give the murexide test; their structure is analogous to that of purines, with substitution of a pyrazine ring for the imidazole ring.



METABOLISM OF NUCLEIC ACIDS

*"All nature is but art unknown to thee;
All chance, direction, which thou canst not see."*

— ALEXANDER POPE

DIETARY PURINES

Pyrimidines and purines are ingested largely in the form of nucleoproteins; only small quantities of nucleotides and free purines or pyrimidines are present in foods. Approximate concentrations of total purines in foods are given in Table 89; the chief dietary sources of purines are meat and fish. The human adult ingests about 0.2 gm. of purine nitrogen daily.

DIGESTION OF NUCLEOPROTEINS

These substances are not digested by saliva. In the stomach, they are partly hydrolyzed by pepsin to a mixture of proteoses and insoluble nucleins (protein-nucleic acid complexes). The nucleins are hydrolyzed by trypsin in the small intestine, and the liberated α -nucleic acid is then depolymerized to β -nucleic acid (tetranucleotide) by nucleogelase (ribonuclease and thymonucleodepolymerase) of the pancreatic juice. The tetranucleotides are hydrolyzed to mononucleotides by nuclease (polynucleotidase) of the succus entericus and intestinal mucosa. Non-specific phosphatases present in bile, pancreatic juice, and the intestinal mucosa digest the mononucleotides to nucleosides and inorganic phosphate; the optimum pH for this reaction is 9.1. Some nucleoside is absorbed unchanged, but a major fraction of the purine nucleoside is converted to purines and sugars by purine nucleosidase of the pancreatic juice and intestinal mucosa. The optimum pH of the purine nucleosidase is 7.5.

TABLE 89

APPROXIMATE PURINE CONTENT OF FOODS

Food	PURINE NITROGEN ¹ (PER CENT)
Sweetbreads (thymus and pancreas) . . .	0.43
Sardines	0.23
Anchovies, herring, kidney, liver, yeast . . .	0.15
Roe, scallops	0.12
Goose, salmon	0.10
Other fish and meats	0.02-0.08
Vegetables ²	0-0.05
Cheese	0-0.02
Cereals, eggs, fruits, and milk	0
	METHYLPURINE (PER CENT)
Tea leaves	2.0 ³
Cocoa	1.5 ⁴
Coffee beans	1.2 ⁵

¹ To estimate the quantities of purine bases and nucleic acids, these figures should be multiplied by 2 and 9, respectively.

² Beans, mushrooms, lentils, peas, and spinach are the only common vegetables which contain more than 0.01 per cent purine nitrogen.

³ As theophylline, although a variable fraction is actually caffeine. Reported values for caffeine in prepared beverages are: coca-cola, 0.025 per cent; cocoa, 0.002 per cent; coffee, 0.087 per cent; tea, 0.036 per cent.

⁴ As theobromine.

⁵ As caffeine.

Digested nucleoprotein is absorbed in the small intestine as a mixture of purines, pyrimidines, ribose, desoxyribose, inorganic phosphate, and nucleosides. The purine nucleosides are absorbed more rapidly than free adenine or guanine; the latter are found in considerable quantities in the excreta of fish-eating birds. Unabsorbed purines are partially destroyed by bacteria in the large intestine.

TRANSPORTATION

The digestion products of nucleoproteins are transported by the blood, but little is known concerning physiological variations of the blood purines and pyrimidines. The erythrocytes contain the following endogenous purine derivatives: cozymase I, about 2 mg. per cent; cozymase II, about 4 mg. per cent; adenylic acid (present as adenosine triphosphate), 65 ± 15 mg. per cent; and some uric acid riboside. The nucleotide concentration of blood varies with the erythrocyte and leukocyte counts. Reticulocytes contain more adenosine triphosphate than the adult erythrocytes, and they have some nucleic acid. The free uric acid (3 ± 1 mg. per cent), which is normally present in blood, is an oxidative catabolite (page 522).

TISSUE PURINES AND PYRIMIDINES

Mammalian tissues contain from 7 to 10 mg. per cent guanine; the scales and skin of bony fishes and the xantholeucophores of frog skin contain larger quantities. Small amounts of paraxanthine are found in mammalian tissues. Only traces of adenine and guanine are present in milk. Intravenously injected purines tend to be toxic; guanine has a histamine-like effect on capillaries. The purine oxidation products, hypoxanthine, xanthine, and uric acid, are widely distributed in tissues. The injection of salts of xanthine and uracil protects the livers of rats against the necrosis and cirrhosis caused by chloroform and carbon tetrachloride; the livers of the protected animals have a high glycogen content. Free pyrimidines have not been detected in normal mammalian tissues, although orotic acid, a pyrimidine derivative, is found in milk.

Guanosine and uric acid riboside are present in many tissues. The extranuclear monoribonucleotides, adenylic and guanylic acid, are also normal tissue constituents; the former is deaminated to inosinic acid by adenylic deaminase, under anaerobic conditions. The cozymases (dinucleotides), and the flavin-adenine dinucleotide prosthetic radical of diaphorase, are found in many cells. Canine liver, kidney, and muscle contain approximately 120, 100, and 50 mg. per cent cozymases, respectively. The liver and muscle cozymase concentrations fall during nicotinic acid deficiency (page 661). The cozymase content of embryonic and malignant tissues is low. By far the greater portion of the purine nitrogen of erythrocytes, brain, kidney, liver, pancreas, spleen, and adult mammalian muscle is present as extranuclear nucleotides; in resting striated muscle they are segregated in the isotropic segments. Purine nucleosides, nucleotides, and nucleic acids stimulate the maturation of the granular cells of bone marrow, and induce peripheral neutrophilic leukocytosis. Hence, injections of adenosine and of adenylic acid have been used in the treatment of agranulocytosis and other leukopenias. Adenosine dilates the coronary arteries, and adenylic acid is beneficial in the glossitis of pellagra and in lip ulcers associated with leukopenia.

Nucleic acids are typical constituents of animal, plant, and bacterial cells. Feulgen's nucleal reaction demonstrates that desoxyribonucleic acids are confined to the chromatin of the nuclei of plant and animal cells, whereas the ribonucleic acids are present in the cytoplasm and the nucleolus. Cytoplasmic nucleoproteins tend to segregate in the high molecular and microscopically visible particles. The concentration of nucleic acid is increased in cytoplasm during rapid growth (as in early embryonic tissue), also in active glands, certain nerve cells, and cells which have been exposed to x-rays. Despite their lack of discrete nuclei, bacteria contain greater concentrations of nucleic acids than do most animal cells. The resting nucleus is a sol, or a gel, enclosed by a nuclear membrane. Its

only definite, microscopically visible structure is the nucleolus. The formation of other nuclear structures during mitosis involves rearrangement, rather than synthesis, of nucleic acids. Nucleoproteins aggregate into discrete chromosomes during the prophase, and they are concentrated in the chromosomal bands or chromomeres. The nucleoprotein mixture which stains as chromatin contains excess nucleic acid. The free acid is stained by basic dyes, whereas that which is fully combined with protein is not stained by these dyes. The nucleoproteins are very important in cellular metabolism, reproduction, and development (page 495).

SYNTHESIS OF NUCLEIC ACIDS AND NUCLEOPROTEINS

The purine, pyrimidine, and carbohydrate units of nucleic acids are essential nutrilites for certain bacteria, but they are not required in the diet of mammals. The only essential pyrimidine derivatives for animals are thiamin and riboflavin (vitamins B₁ and B₂). Cellular nucleoproteins are synthesized readily from endogenous organic substances and dietary inorganic phosphate. In the tissues of plants and animals, the purine and pyrimidine units are synthesized from proteins, and the ribose and deoxyribose from carbohydrate intermediates. Animals incorporate N¹⁵ of administered ammonium salts or amino acids into the purine and pyrimidine units of the cellular nucleic acids very readily. All amino acids except lysine participate in the synthetic process. When salmon migrate from salt to fresh water, they do not consume food; and yet they synthesize large quantities of nucleic acid from the nitrogen of muscle proteins. Developing birds and young mammals, whose foods are purine-free (eggs for the former, and milk for the latter), increase their nucleoprotein content rapidly. The hepatic synthesis of hypoxanthine is accelerated in birds by oxaloacetate and glutamine. Purine-free diets can be administered safely to human beings over long periods of time, provided adequate protein is ingested. In fact, orally administered purines and pyrimidines labeled with N¹⁵ are not incorporated into the purine and pyrimidine units of deoxyribonucleic acid, but are catabolized by mammals.

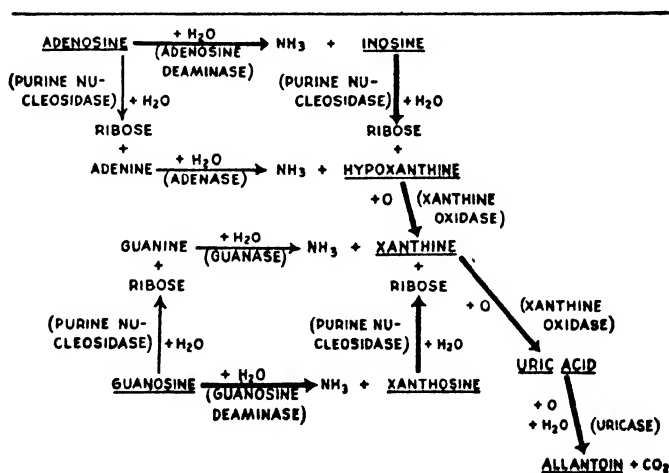
Cellular nucleases, nucleosidases, phosphatases, and other related enzymes cause fragmentation of nucleic acids during autolysis; and, by reverse action, they cooperate in nucleic acid synthesis in living cells. Studies with radioactive phosphate show that approximately 14 per cent of the nucleic acids in muscle and 6 per cent of those in the brain, liver, and thymus are regenerated within twelve hours. The ribonucleic acid turnover exceeds that of deoxyribonucleic acid. The rate of nucleic acid synthesis is controlled by very complicated intracellular mechanisms. When cells differentiate, specialize, or increase in size prior to division, a rapid synthesis of nucleic acid and cysteine occurs. Cells of cancer and

embryonic tissues synthesize greater quantities of nucleic acids than do normal adult tissues, and they also multiply more rapidly. In late fetal life, the purine nitrogen increases more slowly than the protein nitrogen.

CATABOLISM OF NUCLEIC ACIDS, PURINES, AND PYRIMIDINES

Many tissues contain ribonuclease and thymonucleodepolymerase, which catalyze the depolymerization of the high molecular nucleic acids; glandular tissues show the greatest activities. The nucleases of plant and animal cells hydrolyze nucleic acids to mononucleotides; the latter are hydrolyzed by intracellular phosphatases to inorganic phosphate and nucleosides. Animal tissues contain purine and pyrimidine nucleosidases which can liberate purine or pyrimidine bases at an optimum pH of 6.5. The purine nucleosidases of liver have been identified as phosphorylases which catalyze the replacement of the nucleoside nitrogenous unit by phosphate, with the formation of pentose-1-phosphate. Since the reaction is reversible, the nucleoside phosphorylases may be concerned in the synthesis of nucleosides. From 2 to 3 gm. administered adenosine, or guanosine, are metabolized completely by dogs; about 50 per cent of the nitrogen of these nucleosides appears in the urine as allantoin. An additional 30 per cent of the nitrogen of administered guanosine (or guanine) is eliminated as urea.

Intermediate stages of purine oxidation are outlined in Figure 9. While alternate pathways for the oxidative deamination of purine nucleosides to



THE MAIN CHANNEL OF OXIDATION IS INDICATED BY HEAVY ARROWS. THE PRINCIPAL PURINE INTERMEDIATES ARE UNDERLINED.

FIG. 9. Purine deamination and oxidation.

hypoxanthine and xanthine are shown, initial deamination preponderates in mammalian tissues. Under anaerobic conditions, adenosine and muscle adenylic acid are readily deaminated by muscle. The intestine also contains considerable adenosine deaminase. Free purines are not deaminated by muscle, since it does not contain the adenase and guanase found in other tissues. The small quantities of ammonia formed by the deamination of nucleotides, nucleosides, pyrimidines, and purines are transported from the general tissues to the liver and are there converted to urea.

Xanthine oxidase (an aerobic dehydrogenase) oxidizes hypoxanthine and xanthine to uric acid, the chief purine excretory product of insects, snakes, birds, lizards, the Dalmatian hound, anthropoid apes, and man. In the human embryo, xanthine oxidase appears later than the purine deaminases. It occurs mainly in the liver of man, but hepatectomized hounds continue to produce uric acid. The xanthine oxidase activity of the rat's liver is lowered during riboflavin deficiency, and it is subnormal in fetal liver and hepatoma tissue.

In most mammals, uric acid is an intermediate purine metabolite, from 80 to 98 per cent of which is oxidized to allantoin and carbon dioxide by the enzyme, uricase. This enzyme is found largely in the liver, and in small quantities in the kidneys, pancreas, and spleen. It has two pH optima (8.9, and 10.0). Oxygen is essential for uricase activity. Hepatectomy greatly diminishes the urinary allantoin excretion of mammals. Since uricase does not occur in man, he forms little allantoin; neither does human allantoic fluid contain allantoin, although this substance was discovered originally in mammalian allantoic fluid. The local application of allantoin stimulates the healing of wounds. Man can partly destroy administered allantoin. In amphibia and fishes, purine oxidation is carried to the stage of urea by the enzymes, allantoinase and allantoicase. The former oxidizes allantoin to allantoic acid, while the latter converts allantoic acid to urea and glyoxylic acid (page 510). Allantoinase is present in invertebrates and in plants.

In human beings, the dietary methylpurines (caffeine, theobromine, and theophylline) are excreted in the urine partly in unchanged form, and partly as mono- and di-methylpurines and as methyluric acids. A considerable fraction of caffeine is converted to urea.

When small quantities of cytosine and somewhat larger amounts of thymine and uracil are administered to dogs, these substances are excreted principally in the unchanged form; the corresponding pyrimidine nucleosides are converted to urea. Mammals can deaminate these nucleosides and cytosine rather easily, but they oxidize only very limited quantities of free thymine and uracil. The principal end product of mammalian pyrimidine metabolism is urea.

Ribose and desoxyribose are utilized completely only when they are administered as nucleosides or nucleotides.

METABOLISM OF URIC ACID

Uric acid is a characteristic purine metabolite of birds, reptiles, the Dalmatian hound, anthropoid apes, and man; but in birds, snakes, lizards, and invertebrates it replaces urea as the principal catabolite of protein metabolism (uricotelic protein catabolism). Birds lack hepatic arginase, and their livers and kidneys synthesize uric acid from the ammonia produced by amino acid deamination. Extirpation of geese livers interferes with the formation of uric acid and causes the excretion of ammonium lactate. Uricotelic catabolism of protein is characteristic of animals developed in cleidoic systems, such as the terrestrial or closed box type of egg, and the reorganizing insect pupa. In cleidoic eggs, the essential water supply of the embryo is very limited, and insoluble urate crystals are excreted in preference to urea. During the first quarter of development, chick embryo tissues contain uricase; and at this time, ammonia and urea are excreted. Later, the uricase disappears and uric acid is excreted.

The blood of normal fasting human beings contains 3 ± 1 mg. per cent free uric acid, the largest fraction being present in the plasma (Table 73, page 408). The erythrocytes contain uric acid riboside and a variety of non-purine substances which reduce the customary phosphotungstic acid reagent slightly. The true uric acid concentration of blood can be estimated by means of a uricase preparation. About 99 per cent of the plasma uric acid exists as sodium acid urate. Transudates and exudates have approximately the same uric acid content as blood plasma, but cerebrospinal fluid and milk contain only 0.75 ± 0.5 and 1.5 mg. per cent, respectively. Normal tissues contain 2 mg. per cent, or less. The muscles assimilate very little uric acid after intravenous injection, but the kidneys can accumulate as much as 200 mg. per cent. Blood uric acid is not appreciably increased in normal persons by the administration of high purine or high protein diets; in nephritics, similar measures can raise the level from 1 to 2 mg. per cent. The chief factor in the control of the uric acid blood level is the rate of excretion by the kidney. The excretion of uric acid is retarded, and the blood level is raised, by starvation, high fat diets, severe exercise, and the administration of alkali, lactic acid, or adrenaline. The blood uric acid level is lowered by injection of insulin.

Uric acid is the chief end product of exogenous and endogenous purine catabolism in humans. On a purine-free diet, an adult man excretes 0.3 ± 0.1 gm. uric acid daily. Uric acid excretion does not give a quantitative estimate of purine catabolism, since from 30 to 70 per cent of the uric acid which is formed in the body is destroyed (largely in the liver). Individuals vary considerably in their ability to destroy uric acid. Urinary excretion of uric acid usually accounts for only one half of intravenously injected lithium urate, and of ingested hypoxanthine or xanthine. When uric acid is administered by mouth, only 25 per cent is excreted in the

urine, because of poor absorption and destruction of the uric acid by intestinal bacteria. The end products of uric acid oxidation in man are unknown; very little allantoin is produced.

EXCRETION OF PURINE AND PYRIMIDINE METABOLITES

In the human adult, the average daily urinary output of uric acid is approximately 0.7 gm.; a purine-free diet or fasting decreases the daily elimination to 0.3 gm., while high purine diets may raise it to 1.5 or 2.0 gm. The excretion of uric acid (and allantoin) is accelerated by diuretics, cinchophen, salicylates, or thyroid, and by the ingestion of carbohydrate, protein, or purines. Factors which retard the excretion of uric acid have been listed above. Like urea, uric acid is reabsorbed passively in the renal tubules; the kidney normally concentrates urates about twenty times (Table 77, page 434). The clearance of uric acid is approximately one tenth that of urea. About one third of the uric acid of normal urine is present as free acid, and two thirds as the acid urates of ammonium, potassium, and sodium. The free acid fraction decreases to zero at pH 8, and increases to three fourths of the total uric acid at pH 5. In adults, uric acid accounts for approximately 2 per cent of the total urinary nitrogen, whereas in infants it may constitute from 7 to 8 per cent. Infants eliminate from two to four times as much uric acid as do adults per kg. of body weight. The relatively large excretion of uric acid by infants may lead to the formation of crystalline uric acid and ammonium acid urate infarcts during the first few days of life. Such renal deposits can redissolve without permanent injury. A small quantity of uric acid is excreted in the perspiration; no uric acid is found in the feces.

Certain mammals excrete considerable purine base which is not in the form of uric acid. Only from 15 to 60 mg. of such bases are excreted daily by humans; they include 1- and 7-monomethylpurines and paraxanthine, which result from the partial demethylation of the methylpurines of ingested chocolate, cocoa, coffee, and tea. Traces of adenosine, adenine, guanine, hypoxanthine, 7-methylguanine, and xanthine are also present in normal urine, but pyrimidines are not present.

About 30 mg. allantoin are excreted daily in human urine; it is derived largely from exogenous sources. In most mammals, allantoin replaces uric acid as the chief purine metabolite.

PATHOLOGY OF PURINE METABOLISM

"Problems are not exhausted, but men are exhausted in a problem. Fresh talent arriving without a reputation will always find a new aspect." — S. RAMON Y CAJAL

HYPERURICEMIA

Decreased excretion is the principal factor in the pathological elevation of blood uric acid. Hyperuricemia occurs, at times, in glomerulonephritis,

nephrosclerosis, destructive kidney diseases, urinary obstructions, acute intestinal obstruction, hypertension, and congestive heart failure. In glomerulonephritis and eclampsia, the blood uric acid can increase independently of urea or creatinine. In pneumonia, polycythemia, chronic leukemia (particularly the myelogenous type), and in some cases of multiple myeloma, there is increased endogenous nucleoprotein catabolism which results in hyperuricemia and increased urinary excretion of uric acid and other purine bases. During remissions of pernicious anemia, the hematopoietic system becomes increasingly active, and the blood uric acid level rises immediately before the increase in reticulocyte count. This hyperuricemia probably represents increased catabolism of the nuclear constituents of the normoblasts. When relapse occurs, the blood uric acid concentration may become slightly subnormal. The uric acid level of blood is usually normal in liver disease, but acute extensive damage results in hyperuricemia and decreased uric acid excretion. Hyperuricemia is of greatest clinical interest in connection with gout.

GOUT

An important characteristic of the arthritic condition known as gout is the deposition of crystalline sodium acid urate tophi in the tissue spaces of cartilage, tendon and connective tissue of the extremities, and at times in the kidneys and other organs. Tophi in the joints cause inflammation and arthritic symptoms; the tissue irritation is accompanied by partial resolution of the crystals, and by an increased uric acid elimination during the gouty attack. Lead poisoning, high caloric intake, and excessive ingestion of fermented beverages can incite gout; but the condition is partly hereditary. In the gout resulting from lead poisoning, the tophi develop rapidly and become widespread. Pigs can develop a form of gout in which crystalline guanine is deposited as tophi. It is apparently associated with a deficiency of guanase in tissues.

The uric acid level of blood is characteristically elevated in gout. Before, and during, the acute gouty attack, the uric acid level may reach from 6 to 15 mg. per cent. Other nitrogenous constituents of the blood are not increased, unless nephritis is present. In gouty patients, the ability to destroy uric acid partially is not impaired; but high purine diets tend to provoke acute attacks. That hyperuricemia *per se* is insufficient to cause gout is shown by the absence of gouty symptoms in leukemia. Artificial elevation of the blood uric acid concentration does not precipitate gouty attacks, whereas production of diuresis can do so. The urinary uric acid output is often low before an attack; it can be increased by the administration of cinchophen, colchicine, salicylates, or salyrgan. The retention of uric acid during the prodromal period is accompanied by increased elimination of salt and water. The onset of the attack coincides with a marked increase in uric acid excretion.

Treatment of Gout

The purine-free, low calorie diet used in the treatment of gout consists essentially of cheese, eggs, milk, cereals, fruits, and vegetables. These foods provide protein and carbohydrate, which stimulate uric acid excretion. Fats and high purine foods (Table 89, page 517), which tend to raise the blood uric acid level, are eliminated from the diet. Alcohol, chocolate, cocoa, coffee, and tea are also omitted. Exercise is important. The hyperuricemia can often be reduced by the administration of cinchophen, colchicine, salicylates, and the like. Prolonged use of cinchophen can, at times, cause insidious chronic hepatic disease, and large doses of colchicine affect mitosis (pages 210 and 498).

CHEMISTRY OF PORPHYRINS AND RELATED PIGMENTS

"The advance of knowledge is an infinite progression towards a goal that forever recedes." —
SIR JAMES G. FRAZER.

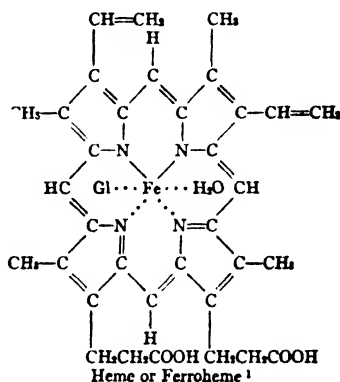
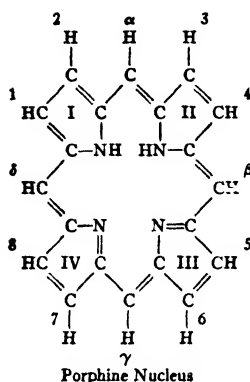
PORPHYRINS

The porphyrin respiratory pigments of animals and plants contain the symmetrical heterocyclic porphine nucleus, which consists of four pyrrol rings united through methene ($-\text{CH}=\text{}$) radicals (Table 90). Porphines exhibit the phenomenon of *mesomerism* or *resonance*, that is, their interatomic linkages exist in an intermediate state which can be portrayed equally well by several arrangements of the unsaturated bonds. There is no real difference between the several pyrrol rings; the two hydrogens, which are indicated as attached to the nitrogens of rings I and II, probably form bridges between pairs of the nitrogen atoms.

The porphine acids, or porphyrins (Table 90), are metabolites of the blood pigments. They have methyl, ethyl, vinyl ($-\text{CH}=\text{CH}_2$), acetic acid, and propionic acid radicals substituted for hydrogens 1 to 8 of the porphine nucleus. Protoporphyrin, the coproporphyrins, and the uroporphyrins have two, four, and eight acid radicals, respectively. The porphyrins dissolve in solutions of alkalis or of highly dissociated acids, in acid alcohol, and ether. The porphyrins of biological interest belong to two series, I and III, which are isomeric in regard to substitutions at atoms 7 and 8. Porphyrins of series III are directly related to the prosthetic radical of hemoglobin; thus, protoporphyrin III unites with ferrous iron to produce the heme radical of hemoglobin. Only traces of protoporphyrin III are found in human feces, but this pigment does occur in erythrocytes, rat feces, avian egg shells, and in plant tissues. Hematoporphyrin is a brown-violet pigment, formed by drastic hydrolysis of hematin in concentrated sulfuric acid, a reaction in which the vinyl side chains of protoporphyrin are hydrated to $-\text{CHOH}-\text{CH}_3$ radicals. Coproporphyrins and uroporphyrins are red excretory products of urine and feces;

when irradiated with ultraviolet light, they show a red fluorescence. The various porphyrins can be differentiated by their absorption spectra, and by the melting points of their methyl esters. Coproporphyrins are soluble

TABLE 90
PORPHYRINS



PORPHINE ACIDS¹
(Iron-free Porphyrins)

	1,3,6	2,4	6	7	8
Protoporphyrin III	—CH ₃	—CH=CH ₂	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₃
Hematoporphyrin III	—CH ₃	—CHOHCH ₃	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₃
Coproporphyrin III	—CH ₃	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₃
Uroporphyrin III	—CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₂ COOH
Coproporphyrin I	—CH ₃	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₃	—CH ₂ CH ₂ COOH
Uroporphyrin I	—CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₂ CH ₂ COOH	—CH ₂ COOH	—CH ₂ CH ₂ COOH

IRON COMPLEXES OF PROTOPORPHYRIN

Divalent Iron Complexes	Radicals on Iron Atom	Corresponding Chromoproteins
Heme (ferroheme, protoheme IX)		Hemoglobin (ferrohemoglobin), myoglobin, erythrocyruorin, heliocorubin, actinohematin
Oxyheme (oxyferroheme)		Oxyhemoglobin, and oxidized forms of other pigments listed above
Carbonylheme (carbonylferroheme)		Carbonylhemoglobin (carbon monoxide hemoglobin)
Sulfoheme (?)	Structure not known, contains =S radical	Sulfohemoglobin
Trivalent Iron Complexes		
Metheme (hematin, ferriheme hydroxide, protoferriheme IX)		Methemoglobin (ferrihemoglobin), catalase, peroxidases

TABLE 90 (Cont.)

PORPHYRINS

IRON COMPLEXES OF PROTOPORPHYRIN		
Trivalent Iron Complexes	Radicals on Iron Atom	Corresponding Chromoproteins
Hemin (ferriheme chloride)	$ \begin{array}{c} \text{N} \quad \text{N} \\ \diagdown \quad \diagup \\ \text{Protein} \cdots \text{Fe} - \text{Cl} \\ \diagup \quad \diagdown \\ \text{N} \quad \text{N} \end{array} $	
Ferriheme cyanide	$ \begin{array}{c} \text{N} \quad \text{N} \\ \diagdown \quad \diagup \\ \text{Protein} \cdots \text{Fe} - \text{CN} \\ \diagup \quad \diagdown \\ \text{N} \quad \text{N} \end{array} $	Cyanhemoglobin

IRON COMPLEXES OF OTHER PORPHYRINS		
	Structural Relation to Heme	Corresponding Chromoproteins
<i>Spirographis</i> Heme	Formyl Radical at Position 2	Chlorocruorins
α -Heme	One Vinyl Radical Substituted in unknown fashion	Cytochrome c
Pheohemin ⁴	Related to <i>Spirographis</i> Heme	Cytochrome Oxidase

MAGNESIUM COMPLEXES OF PORPHYRINS ⁵					
	5	4	3 to 6	7	8
Chlorophyll a		—CH ₂ CH ₃	—CHCO—	—CH ₂ CH ₂ COO—Phytyl ⁶ and —H	—CH ₃ and —H
			COOCH ₃		
Chlorophyll b	—CHO	—CH ₂ CH ₃	—CHCO—	—CH ₂ CH ₂ COO—Phyty and —H	—CH ₃ and —H
			COOCH ₃		

¹ The free heme does not contain the radicals, H₂O and Gl (globin), but these are present in the molecule of hemoglobin.

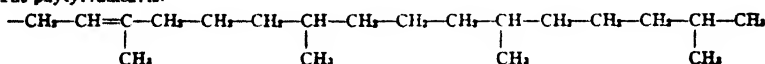
² These porphyrins have structures similar to that given for heme, with the substitutions noted in the table. The III series of porphyrins have their substituents in the same relative order as in heme, while the porphyrins of the I series are isomers with the substituent radicals at positions 7 and 8 in reverse order.

³ In catalase, H₂O₂ can occupy this position.

⁴ The word pheohemin means dark hemin; it is sometimes applied to *Spirographis* heme.

⁵ These porphyrins have structures similar to heme, with the exceptions noted in the table and the substitution of Mg for Fe. The chromoprotein compounds of the chlorophylls are called chloroplastins. Bacteriochlorophyll is similar to chlorophyll a except that the vinyl radical at position 2 is replaced by a CH₂CO— radical.

⁶ The phytol radical is:



in a mixture of acetic acid and ether, whereas uroporphyrins are not. Coproporphyrin I, and a trace of uroporphyrin I, are excreted normally in bile, feces, and urine; the corresponding III isomers are excreted in certain pathological porphyrias. These substances form colorless porphyrinogens, which can be converted to the porphyrins by oxidation with iodine or permanganate, or by exposure to light and air. Coproporphyrin I is present in eggs and in yeast; coproporphyrin III is formed by bacteria and plants. Turacin, a pigment of bird feathers, is a copper salt of uroporphyrin I. Uroporphyrin III has been reported in the feathers of many species of tropical birds. Porphyrins, of fossil origin, are found as vanadium complexes in asphalt, coal, and petroleum.

METAL-PORPHYRIN COMPLEXES

The most important prosthetic porphines of animal and plant pigments are the metal-porphyrin complexes. The chlorophylls of green plant tissues and of green and purple bacteria contain magnesium, while the prosthetic radicals of hemoglobin and related chromoproteins are iron-porphyrin complexes (Table 90). These pigments are coordination compounds in which primary and secondary valences of the metal (indicated in the table by — and . . ., respectively) complete the internal cyclic structure of the porphyrin complex. The modern nomenclature of biological iron-porphyrin complexes is given in the table. These prosthetic pigments are insoluble in water, but can be dissolved in aqueous alkali and in acid alcohol solutions.

When one of the valences of the heme iron atom is combined with a nitrogenous radical of a protein (probably a histidine unit), the metal-porphyrin complex acquires its biologically important property of combining reversibly with molecular oxygen. Heme combines with protein through a residual valence linkage, which is easily dissociated by acids and alkalis, or by denaturation of the protein. The protein and the four porphine nitrogens satisfy five of the six iron valences; in hemoglobin the sixth linkage is with water, to form an aquo complex (Table 90). Heme, hemoglobin, oxyhemoglobin, and carbonylhemoglobin contain ferrous iron, and hence two of the iron linkages represent primary valences. Replacement of the H_2O radical of hemoglobin by O_2 or CO gives oxyhemoglobin or carbonylhemoglobin, respectively. Hematin, hemin, methemoglobin, and cyanhemoglobin contain ferric iron. Free heme is very rapidly oxidized to hematin, and the latter can be reconverted to heme only by the action of a strong reducing agent, such as sodium hyp-sulfite ($\text{Na}_2\text{S}_2\text{O}_4$). The structure of sulfhemoglobin is not definitely known.

Iron-containing ions of the heme complexes show *paramagnetic susceptibilities*; when electrons enter the atomic shells and form covalent linkages, the iron-porphyrin complexes become diamagnetic. Studies of magnetic susceptibilities indicate that the iron valences in heme, hemoglobin, hematin, hemin, methemoglobin, catalase, and peroxidase are ionic in character, while those in oxyhemoglobin, carbonylhemoglobin, cyanhemoglobin, and cytochrome *c* are covalent. The dissociation constants, isoelectric points, and molecular weights of chromoproteins are given in Tables 1, page 4; 10, page 47; and 71, page 397, respectively.

Porphyrin pigments can be conveniently identified by their absorption spectra. The positions of the characteristic α and β absorption bands are given in Table 91. Porphyrin compounds also have γ bands in the violet region of the spectrum, which have much higher extinction coefficients than the bands listed in the table. Ferrous porphyrin-protein complexes, whose iron atoms have a covalent linkage, show two prominent absorption bands between 500 and 600 $\text{m}\mu$, while those with ionic linkages

TABLE 91
CHARACTERISTIC ABSORPTION BANDS OF PORPHYRINS^{1,2}
(Approximate Centers of Bands)

D line of Na	Red	Orange	Yellow	Green	Blue	Violet
	700	650	600	550	500	450
<i>Chromoproteins</i>			589			
Carboxyhemoglobin				570		
Carboxymyoglobin		629		579		
Catalase				542		
Cytochrome c				540		
Cytochrome c peroxidase (reduced)				556		
Cytochrome c peroxidase (oxidized)				570		
Chlorocruorin		630	603			
Chloroglobin						
Cyanhemoglobin				552		
Cytochrome (reduced) ³				566-580c		
Cytochrome (oxidized)				567		
Cytochrome c peroxidase (reduced)				560		
Cytochrome c peroxidase (oxidized)		620	605a			
Deoxyhemoglobin				559		
Deoxymyoglobin		634	582	559		
Methemoglobin (acid)		631	576	548		
Methemoglobin (alkaline)			592	555		
Myoglobin			579	542		
Oxyhemoglobin			575	540		
Peroxidase I		640				
Peroxidase II		637				
Sulfhemoglobin		617	578	540		
Verdoperoxidase (reduced)	690	625		570		
Verdoperoxidase (oxidized)					475	
<i>Porphyrias</i>						
Bacteriochlorophyll a	680		584			
Bacteriochlorophyll b		670	579			
Chlorophyll a		658				
Chlorophyll b		665	610			
Coproporphyrin I (acid)						
Hematin (acid)		638				
Hematin (alkaline)						
Hematoporphyrin (acid)		623	578	568		
Hematoporphyrin (alkaline)			605	535		
Heme			596	534		
Protoporphyrin (acid)			615	552		
Protoporphyrin (alkaline)			607	572		
Uroporphyrin (acid)			602	536		
Uroporphyrin (alkaline)			597	557		
Urobilin		625	577	567		

¹ The α bands are to the left.

² The wave lengths are given in m μ . (10 Å.)

³ The symbols "a," "b," and "c" indicate the α bands for cytochromes a, b, and c, respectively; the wave length, 521 m μ , is the β band for all cytochromes.

have only one band in this portion of the spectrum. The spectroscopic detection of carbonylhemoglobin, methemoglobin and sulfhemoglobin is of clinical interest. Carbonylhemoglobin has α and β absorption bands which are similar to those of oxyhemoglobin, but they are shifted slightly to the right. A comparison spectroscopy is necessary to detect this displacement. However, oxyhemoglobin and methemoglobin can be rapidly changed to hemoglobin by the addition of sodium hyposulfite or of a freshly prepared ammonium ferrotartrate solution (Stoke's reagent), whereas carbonylhemoglobin is not affected and its absorption spectrum is not changed by these reagents. The characteristic absorption band of methemoglobin disappears on the addition of Stoke's reagent, ammonium sulfide, or potassium cyanide solution. Sulfhemoglobin is not reduced by these reagents, but its characteristic absorption band shifts slightly to the right when the solution is treated with carbon monoxide. The methemoglobin spectrum is not changed by carbon monoxide. The detection of abnormal chromoproteins in blood by an ordinary spectroscopy has very definite limitations; methemoglobin must constitute about one fourth of the total blood pigment to be recognized easily.

Hemoglobin

This pigment is the reduced form of the erythrocytic chromoprotein of vertebrates. It is a complex which consists of one molecule of globin and four molecules of heme. Hemoglobin contains 3.7 per cent heme, and 0.34 per cent iron. The hemoglobins are species specific because they have globin radicals of different compositions. When hemoglobin is warmed gently with glacial acetic acid and sodium chloride, it dissociates into globin and free heme, and the latter is oxidized to hematin. The —OH radical of the hematin is then substituted by —Cl, with the formation of characteristic brown hemin crystals which can be readily identified microscopically. The reaction is termed the *hemin* or *Teichmann test*; it is used to detect blood in fluids, clots, or stains. A more sensitive test for hematin, and for traces of blood, is the *chemiluminescence* reaction. The unknown is mixed with an alkaline solution of 3-aminophthalic hydrazide and a small quantity of hydrogen peroxide, and the mixture is examined in a dark room for the appearance of a blue luminescence.

The *benzidine*, *guaiac*, and *tolidine color tests* for blood are preferred in clinical studies. When hemoglobin is mixed with a glacial acetic acid solution of one of these reagents, and a small quantity of hydrogen peroxide solution is added, a green to blue color develops. Hematin catalyzes the decomposition of hydrogen peroxide to active oxygen, which oxidizes the reagents to blue colored compounds. An excess of hydrogen peroxide must be avoided, since it tends to bleach the blue color and to destroy the hematin. Ascorbic acid also bleaches the blue color of the benzidine reaction. The guaiac test can detect 0.1 mg. per cent of blood in biological

fluids; this test is given by respiratory pigments which contain heme, hematin or *Spirographis* heme. The benzidine reagent is used widely in clinical laboratories. For hemoglobin detection, the sample should first be boiled for fifteen seconds; otherwise, the peroxidases of pus, tissues, and body fluids will give the reaction (page 99). A confirmatory procedure is to hydrolyze the hemoglobin with acetic acid, and to extract the hematin with ether. The evaporated extract is used for the colorimetric tests. The *phenolphthalein* test is relatively specific for hemoglobin, and it is sensitive to 0.1 mg. per cent of this chromoprotein. When the alkaline phenolphthalin reagent is warmed with the boiled sample and a solution of hydrogen peroxide, a red coloration appears if hemoglobin is present. These tests cannot differentiate between the bloods of different species.

Total hemoglobin of blood may be determined quantitatively by the spectrophotometer; but the usual clinical procedure is to convert the hemoglobin to hematin by adding acid, and to compare the hydrolyzate colorimetrically with a standard hematin solution, or with an artificial standard. Photoelectric colorimeters are especially convenient for routine determinations of hemoglobin.

Oxyhemoglobin

In human erythrocytes, both hemoglobin and oxyhemoglobin exist as potassium salts. The oxyhemoglobins of the dog, horse, and guinea pig crystallize readily; the crystal forms of oxyhemoglobins vary with the species. The purple-red solution of hemoglobin combines readily with oxygen to form red oxyhemoglobin, and the reaction is reversed when the oxygen tension is lowered. This reversible reaction is usually represented by the equation, $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$, although four molecules of oxygen actually combine with one molecule of hemoglobin, since the latter has four heme prosthetic radicals. The affinity of hemoglobin for oxygen is decreased on the acid side of the isoelectric point. The dissociation of oxyhemoglobin to hemoglobin is, therefore, accelerated by lowering the pH, and the oxygenation of hemoglobin is accelerated by alkalinity. Hemoglobin becomes saturated with oxygen at an oxygen partial pressure of 150 mm. of mercury.

The oxygen capacity of blood is an index of its hemoglobin content; one gm. of hemoglobin combines with 1.35 ml. of oxygen. The oxygen content, and the oxygen capacity of blood, can be determined in the Van Slyke manometric apparatus. For the determination of the total oxygen content, the blood must be collected under oil (to avoid oxygenation of the hemoglobin by air); but when it is desired to determine the oxygen capacity, the blood is first equilibrated with air. The blood sample is mixed, in the apparatus, with potassium ferricyanide-saponin reagent (which hemolyzes the erythrocytes, and oxidizes the hemoglobin iron to the ferric state). The blood gases are then extracted under diminished

pressure, and the carbon dioxide is removed by absorption in sodium hydroxide solution. The remaining gas is corrected for its nitrogen content; or the oxygen can be determined by difference, after removing it from the mixture by shaking with an alkaline solution of sodium hyposulfite, or pyrogallol.

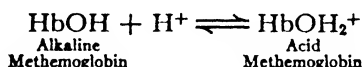
Carbonylhemoglobin

Carbon monoxide combines readily with hemoglobin, and it replaces the oxygen radical of oxyhemoglobin to produce cherry red carbonylhemoglobin. Since hemoglobin has an affinity for carbon monoxide which is two hundred and ten times its affinity for oxygen, the hemoglobin combines preferentially with carbon monoxide even at very low partial pressures. The reaction is reversible, and dissociation of the carbonylhemoglobin can be accelerated by increasing the oxygen tension. Blood samples for the determination of carbonylhemoglobin must be taken as soon as possible, because the carbon monoxide is aerated from the blood within a few hours after the patient is removed to fresh air. The pigment can be determined spectroscopically (page 530); or colorimetrically, by diluting the blood with distilled water, adding a mixture of pyrogallol and tannic acid, and comparing with standards prepared from blood which has been saturated with carbon monoxide gas. Gasometric determination in the Van Slyke apparatus is more exact. In this procedure, the carbon monoxide is liberated by the addition of an oxidizing reagent which contains lactic acid and considerable ferricyanide. Oxygen and carbon dioxide are removed from the gaseous mixture by absorption in alkaline pyrogallol solution. The residual gas is measured and corrected for its nitrogen content, or the carbon monoxide can be determined by difference after absorbing it in Winkler's reagent (an ammoniacal solution of cuprous and ammonium chlorides). Normal human blood contains 0.15 ± 0.05 vol. per cent of carbon monoxide.

Methemoglobin

This is a brown pigment which is produced very slowly in shed blood, but more rapidly in the presence of certain oxidizing agents. Normal human blood contains only 70 ± 60 mg. per cent of methemoglobin. The administration of acetanilide, aminophenols, aniline, antipyrin, chlorates, ferricyanides, hydrogen peroxide, hydroquinone, iodine, nitrites, nitrobenzene, nitrophenols, permanganates, phenacetin, pyrogallol, sulfonamide drugs, sulfonal, trional, or large quantities of methylene blue to animals causes the appearance of appreciable quantities of methemoglobin in the blood and urine. Some of these substances (aniline, acetanilide, phenols, etc.) are reducing agents which act as oxygen carriers in the production of methemoglobin *in vivo*. Hemoglobin is convertible to

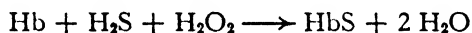
methemoglobin, but oxyhemoglobin is not. The formation of methemoglobin involves the oxidation of the ferrous heme prosthetic radical to the ferric hematin radical, whereas the formation of oxyhemoglobin is an oxygenation or formation of a coordination compound of the heme radical and molecular oxygen. Methemoglobin is slowly converted to oxyhemoglobin by exposure to oxygen; it does not combine directly with oxygen or with carbon monoxide, nor does it dissociate into oxygen and hemoglobin under reduced pressure. However, methemoglobin can be reduced to hemoglobin by sulfhydryl compounds. The equilibrium between the acid and alkaline forms of methemoglobin may be represented by the equation:



Cyanhemoglobin and Sulfhemoglobin

Cyanides convert methemoglobin into the red colored pigment, cyanhemoglobin. Cyanides do not react with hemoglobin or with oxyhemoglobin.

Sulfhemoglobin exists in reduced and oxidized forms. It can be formed from hemoglobin by the combined action of sulfides and hydrogen peroxide.



The reaction is not reversible. The sulfur atom of sulfhemoglobin does not occupy the same position as the carbonyl radical in carbonylhemoglobin, inasmuch as carbon monoxide can combine with sulfhemoglobin. The administration of sulfone derivatives, such as sulfonyl, trional, and the sulfonamide drugs, occasionally causes the formation of sulfhemoglobin *in vivo*.

Hemochromogens and Other Porphyrin Chromoproteins

Heme complexes of organic nitrogenous substances are termed hemochromogens; but the term is used more specifically by biochemists to designate heme chromoproteins which do not contain globin, as for example, denatured hemoglobin (cathemoglobin), whose protein moiety is denatured globin. The *erythrocruorins*, or hemochromogens of invertebrates, contain non-globin proteins which resemble those present in the chlorocruorins. In a few species, the erythrocruorins occur in erythrocytes; more frequently, they are in solution in the body fluids. Other red hemochromogen respiratory pigments are: the heliocorubin of crayfish and molluscs, the actiniohematin of certain *actinia*, and the *myoglobins* of muscle. Myoglobins have lower molecular weights than do the hemoglobins (Table 71, page 397); they combine more readily with oxygen, less readily with carbon monoxide. Carbonylmyoglobin dissociates more easily than car-

bonylhemoglobin. The myoglobins are species specific. The protein moiety of myoglobin resembles the globins (of hemoglobins), but it tends to be polydisperse. Hemoglobins and myoglobins differ immunologically and in their amino acid composition.

Certain porphyrin chromoproteins contain prosthetic radicals which are derivatives of heme (Table 90, page 526). Catalase and peroxidase are protein complexes of hematin; they may be classified with methemoglobin as *parahematin*s. The molecule of catalase contains four iron atoms, which constitute 0.1 per cent of the chromoprotein. Two to four of the iron atoms are present as hematin in the several catalases; the remainder may represent verdohematin, or a similar pigment (page 537). Catalase probably activates the decomposition of hydrogen peroxide by forming an intermediate peroxide. Catalase and the peroxidases combine reversibly with azide, cyanide, fluoride, sulfide, and nitric oxide; these substances, therefore, inhibit catalase and peroxidase activities. Carbon monoxide does not have this effect. The peroxidases form active green complexes with hydrogen peroxide; they contain 0.1 per cent of iron, which apparently remains in the ferric state during oxidation of polyphenols. Lactoperoxidase and peroxidase II have been crystallized; verdoperoxidase is a green parahematin isolated from leukocytes.

The green *chlorocruorins*, found in the plasma of marine worms, contain *Spirographis* heme. The prosthetic radical of *cytochrome oxidase* is a somewhat similar iron-porphyrin complex, termed *pheohemin*. The activity of cytochrome oxidase is inhibited by combination with carbon monoxide, at tensions of 1 atmosphere or more; the enzyme reacts more readily with azides, cyanides, and sulfides, and is inhibited also by ribonuclease. *Cytochrome c* has as its prosthetic radical a *c*-hemin, in which the vinyl side chain has been substituted in an undetermined manner (two vinyl side chains are probably joined through thioether linkages to cysteine units of the protein). Two histidine units of the protein are coordinated with the iron atom of the *c*-hemin. The porphyrin radical of cytochrome *c* seems to be linked to the protein by a primary valence; this linkage is very stable. Hydrolysis of cytochrome *c* ruptures the polypeptide chain, and a portion of the latter remains attached to the porphyrin radical. Cytochrome *c* has an especially high isoelectric point (pH 10.65), and it contains 0.43 per cent of iron. It is oxidized by ferricyanide, and reduced by adrenaline, ascorbic acid, and cysteine. At the pH range of tissues, the cytochromes do not combine with carbon monoxide or with cyanide. (See pages 97 and 100 for further details of cytochromes and cytochrome oxidase.)

NON-PORPHINE CHROMOPROTEINS

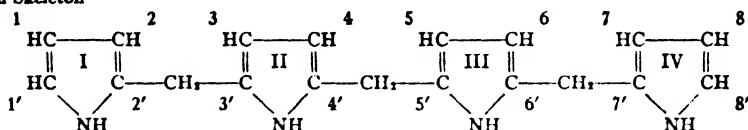
The red *hemerythrins*, in the blood of worms, contain a non-porphine iron prosthetic radical of undetermined composition, which is known as *hemoferrin*.

The crystalline *hemocyanins*, of molluscs and crustaceans, contain from 0.17 to 0.38 per cent of copper. These respiratory pigments are blue in the oxidized condition, and colorless when reduced. The copper atom remains in the cuprous condition during the oxidation-reduction cycle. The hemocyanins are plasma proteins of very high molecular weight (Table 71, page 397). Their copper-containing prosthetic radical, hemocuprin, combines with oxygen or carbon monoxide in the proportion of one mol of gas to two atoms of copper. The hemocyanins have only one fourth the oxygen-carrying capacity of hemoglobins.

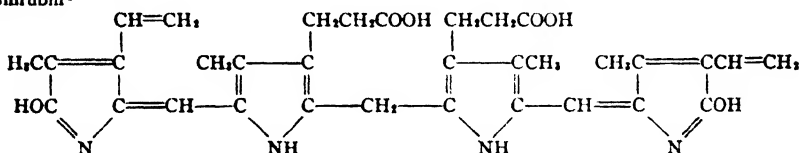
A blue, copper-containing protein, *hemocuprein*, accounts for all of the copper present in mammalian erythrocytes. It has a comparatively low

TABLE 92
BILE PIGMENTS

Bilan Skeleton¹



Bilirubin¹

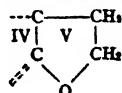


CLASSIFICATION²

	Radicals at			
	1', 8'	I	2	8
Bilans (no methene bridges)				
Mesobilinogen			-C ₂ H ₅	-C ₂ H ₅
Urobilinogen (stercobilinogen)	-H	-H	-C ₂ H ₅	-H
	-OH	-CH ₃	-C ₂ H ₅	-C ₂ H ₅
Bilens (1 methene bridge at 5')				
Urobilin (stercobilin)	-H	-H	-C ₂ H ₅	-H
	-OH	-CH ₃	-C ₂ H ₅	-C ₂ H ₅
Bilidiens (2 methene bridges at 2', 7')				
Bilirubin				
Biliverdins (3 methene bridges)				
Biliverdin (methene bridges at 2', 5', 7')				
Bilicyanin (methene bridges at 3', 4', 6')				

¹ Rings I, II, III, and IV of the bilan skeleton correspond to rings II, III, IV, and I of the porphine skeleton, respectively.

² The radicals at positions 8 and 8' are believed to exist as a fifth ring:

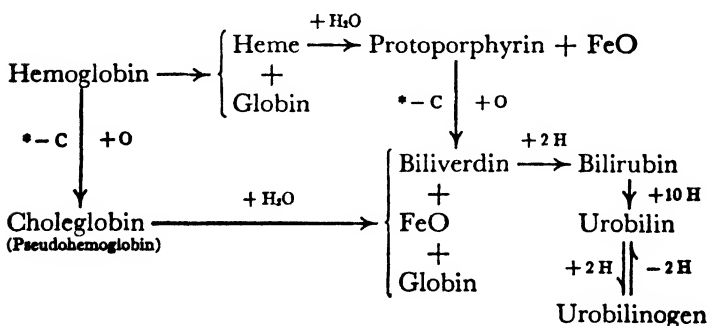


³ The bile pigments have formulae similar to that of bilirubin, with the exceptions noted in the table.

molecular weight, and contains two atoms of copper per molecule. A portion of the copper of mammalian liver is present as a similar protein, *hepatocuprein*. The cupreins have 0.34 per cent of copper. The *polyphenol oxidases* are enzymatic proteins, which have copper-containing prosthetic radicals of unknown constitution. They are yellow or blue chromoproteins, which combine with carbon monoxide, cyanide, and sulfide, and are inactivated by these substances, and by *p*-aminobenzoate and sulfonamides. The copper content of the polyphenol oxidases is from 0.2 to 0.34 per cent. The cupric forms of the enzymes are reduced by the substrates and reoxidized by molecular oxygen. Ascorbic oxidase contains 0.25 per cent copper.

BILE PIGMENTS

The bile pigments are oxidation products of the porphyrins. They are formed by removal of the α -methene ($-\text{CH}=\text{}$) radical which connects porphine rings I and II (Table 90, page 526). The *bilan* skeleton, in which the three remaining methene bridges have been saturated, is shown in Table 92. Urobilinogen and mesobilinogen are bilan derivatives; the other common bile pigments may conveniently be classified as biliens, bilidiens, and bilitriens, according to the number of unsaturated methene bridges which they contain. The most important bile pigments are derivatives of type III porphyrins. In order of increasing saturation, they are: biliverdin, bilirubin, urobilin, and urobilinogen. The last three can be formed from biliverdin by progressive reduction (addition of hydrogen). The relations of the bile pigments to hemoglobin may be illustrated as follows:



The methene bridge between rings I and II is removed in the reactions marked with an asterisk. Removal of the iron atom of heme before oxidation gives rise to protoporphyrin, whereas its removal after oxidation yields biliverdin. The bile pigments are dibasic acids; they are insoluble in water, but soluble in alcohol and to a smaller extent in chloroform.

The alkali salts of the bile pigments dissolve in water; polyvalent cations form insoluble bile pigment salts.

Biliverdin is a green pigment found in bile and (in small amounts) in mammalian tissues. It can be reduced to bilirubin by cellular and bacterial enzyme systems, or by the action of ammonium sulfide. In animals, the heme radical of hemoglobin can be oxidized to a biliverdin-iron complex, verdohematin. The green chromoprotein which contains this prosthetic radical has been termed pseudohemoglobin, verdohemoglobin, or *choleglobin*. Its formation from hemoglobin can be induced by hydrogen peroxide, and it can be formed from oxyhemoglobin by the action of ascorbic acid. The iron atom of verdohematin is removed readily by acids, with the production of biliverdin. A similar iron-containing bile pigment may constitute one of the prosthetic radicals of catalase (page 534). Other bile pigment chromoproteins include the blue phycocyan and red phycoerythrin of algae, which contain prosthetic radicals related to mesobilirubin, and the oocyan of egg shells, whose prosthetic radical is biliverdin. Pterobilin, an isomer of biliverdin, is a blue pigment of butterfly wings.

Bilirubin is a red-brown reduction product of biliverdin; it is found in bile and tissue fluids. This pigment was formerly termed hematoidin. When bilirubin is treated with fuming nitric acid, its vinyl side chains are reduced to ethyl radicals and yellow mesobilirubin is formed. The latter is then oxidized, in rapid succession, to green-blue mesobiliverdin (glauco-bilin), violet-blue mesobilicyanin (mesobilipurpurin), and pink choletelin. This series of reactions is known as *Gmelin's test*; it is used to detect bile pigment in biological fluids. The nitric acid is usually stratified below the test solution, and the play of colors is observed at the zone of contact. Gmelin's test can be performed on strips of filter paper soaked in the unknown, or the bile pigments can be concentrated by adsorption on talc before the nitric acid treatment. The last named procedure (Naumann's method) will detect 1 γ per cent of bilirubin. Bilirubin can be oxidized to green biliverdin by the action of potassium chlorate in acid solution (Huppert's test), or by ferric chloride (Kapsinow's test). Alkaline solutions of bilirubin, in contact with air, oxidize spontaneously to biliverdin.

Bilirubin can be determined quantitatively, as red-violet azobilirubin, by its reaction with freshly prepared Ehrlich's diazo reagent (a mixture of sulfanilic acid, hydrochloric acid, and sodium nitrite solutions). This diazotization is known as the *Van den Bergh reaction*. The serum is first mixed with the reagent; ammonium sulfate is then added to flocculate the plasma proteins, and alcohol is added to liberate and dissolve the bilirubin. The supernatant solution is compared colorimetrically with a similarly diazotized solution of 0.4 mg. per cent bilirubin, or with a 2.161 per cent solution of anhydrous cobalt sulfate. To avoid marked loss of bile pigment during the flocculation of the proteins, the serum should be diluted to a bilirubin content of approximately 3 mg. per cent.

Normal bile, and the biological fluids obtained from patients with obstructive jaundice, give the Van den Bergh reaction without the addition of alcohol and ammonium sulfate; this is termed a *direct* reaction. Normal biological fluids, other than bile, require the addition of alcohol and ammonium sulfate for the color development, and their reaction is said to be *indirect*. Sera can be tested qualitatively by stratifying them above the reagent; a direct reacting serum gives a color at the zone of contact within 10 minutes. The direct reacting form of serum bilirubin is termed *cholebilirubin*, and the indirect reacting form, *hemobilirubin*. Dilute aqueous solutions of sodium bilirubinate give a direct reaction, but as much as 12 mg. per cent of sodium bilirubinate must be added to normal serum to allow a direct reaction. Both forms of serum bilirubin are bile pigment complexes of serum albumin; hemobilirubin is a more stable complex than cholebilirubin. The addition of surface reactive substances (bile salts, soaps, cholesterol, etc.) to normal plasma converts hemobilirubin to cholebilirubin.

Another clinical method of estimating the serum bilirubin concentration is the *icteric index*, which is determined by colorimetric comparison of suitably diluted serum, from unhemolyzed blood, with a 0.01 per cent solution of potassium dichromate. Erroneous values result from hemolysis or abnormal concentrations of carotenoid pigments. Blood samples for the icteric index should not be taken after meals, since the serum may be opaque from the postprandial lipemia. The normal icteric index is 5 ± 1 (i.e., five times the color of the dichromate standard).

UROBILIN, UROBILINOGEN, AND UROCHROME

Urobilin (stercobilin) is a brown reduction product of bilirubin (Table 92). This pigment is responsible for the normal color of feces. Urobilin can be salted out from its aqueous solution by saturation with ammonium sulfate. Urobilinogen (stercobilinogen) is a colorless reduction product of urobilin, which is excreted in the feces and urine. It can be formed from urobilin by the reducing action of ferrous hydroxide. Urinary urobilinogen is oxidized to urobilin on exposure to light and air. Urochrome is a compound of urobilin and a polypeptide; it is the chief pigment of normal urine. Its colorless precursor, urochromogen, is a reduction product of urochrome.

Urobilinogen reacts with Ehrlich's aldehyde reagent (a hydrochloric acid solution of *p*-dimethylaminobenzaldehyde) to produce a red pigment. Normal urine gives a visible coloration with this reagent, in dilutions of 1 to 20 (Wallace and Diamond test). The reaction is the basis of Watson's quantitative colorimetric method for urobilinogen. Ammoniacal solutions of urobilin and urochrome give a green fluorescence upon the addition of zinc chloride.

METABOLISM OF PORPHYRINS

"Far from being discouraged before the great authorities of science, the novice in investigation ought to know that his destiny, by a cruel but inexorable law, is to grow a little at the cost of the reputation of the great authorities." — S. RAMON Y CAJAL

FOOD PORPHYRINS AND HEMATOPOIETIC FACTORS

The chief food porphyrins are the hemoglobins, the myoglobins of meat, and the chlorophylls of vegetables. These substances are not dietary essentials of mammals. Hemoglobin synthesis is dependent on an adequate intake of such non-porphine hematopoietic substances as iron and copper salts, the thermostable extrinsic factor, the erythrocyte-maturing factor, and vitamins B₂ (riboflavin), B₆ (pyridoxin), folic acid, ascorbic acid, and vitamin D. Studies of hemoglobin regeneration in hemorrhagic dogs indicate that foods may be arranged in the order of hematopoietic efficiency shown in Table 93. Some animal foods, such as liver, casein, and oxyhemoglobin, are valuable because of their protein content. Thus, 35 per cent of ingested globin and 13 per cent of liver protein can be converted into hemoglobin. Nuts, leafy vegetables, fruits, liver, spleen, and oysters are excellent sources of iron and copper. Beef and autoclaved yeast are the best sources of the heat-stable extrinsic factor; smaller quantities are present in egg white, wheat germ, and liver. The liver, kidney, and brain contain the erythrocyte-maturing factor. Uncooked gastric and intestinal mucosae can provide the enzymatic intrinsic factor, which is secreted by the normal gastro-intestinal mucosae, but is absent in pernicious anemia. The action of this enzyme on the extrinsic factor produces the erythrocyte-maturing factor.

TABLE 93

HEMATOPOIETIC EFFICIENCY OF FOODS
IN HEMORRHAGIC ANEMIA¹

BEST	INTERMEDIATE	SLIGHT	NEGLECTIBLE
Liver	Pancreas	Spinach	Milk
Kidney	Beef	Fish	Bread
Gizzard	Spleen	Asparagus	Carrots
Eggs	Raisins	Lettuce	Cereals
Apricots	Grapes	Butter	Celery
Peaches	Apples	Lean pork	Berries
Oysters	Prunes	Cheese	
	Brain		

¹ In dogs.

DIGESTION, ABSORPTION, AND TRANSPORTATION

The globin of oxyhemoglobin is digested by pepsin and trypsin. The oxyheme radical of oxyhemoglobin is converted to hematin in the digestive tract. In the reduced form, hemoglobin is digested with difficulty; its presence in normal feces can be shown by the benzidine and guaiac tests. For this reason, patients should be placed on meat-free diets if the feces are to be tested for intestinal bleeding. Hematin and chlorophyll, and their digestion products, are poorly absorbed by the intestine. Chlorophyll is excreted in the feces, partly in unchanged form and partly as pheophytin and phorbides. Ingested protoporphyrin, bilirubin, urobilin, and urobilinogen are only partially absorbed in the intestine. The bile pigments and coproporphyrins are removed rapidly from the portal circulation by the liver and excreted in the bile. In the intestinal lumen, biliverdin is progressively reduced to bilirubin, urobilin, and urobilinogen by bacteria; hemoglobin is quite resistant to bacterial putrefaction. The chief pigment of normal feces is urobilin, although some urobilinogen, coproporphyrin I and other porphyrins are also present (page 548).

Normal blood plasma contains about 0.5 ± 0.3 mg. per cent bilirubin (hemobilirubin), traces of urobilinogen, and, perhaps, other porphine metabolites. A minute quantity of coproporphyrin I is present in human fetal blood. Normal erythrocytes contain approximately 34 per cent hemoglobin, 27 γ per cent protoporphyrin III (12 ± 4 γ per cent in whole blood), and traces of choleglobin and biliverdin. None of these constituents, or the traces of free heme present in tissues, can be regarded as transport forms of absorbed porphine compounds.

SYNTHESIS OF TISSUE PORPHYRINS

All living cells contain porphine derivatives, which are readily synthesized from non-porphine precursors (glycine, glutamic acid, proline, hydroxyproline, and carbohydrate). Porphyrins appear in embryonic tissues very early in development. Normal animal tissues, bacteria, and yeast can synthesize protoporphyrin III and coproporphyrin I. The bone marrow is the chief adult tissue concerned in the synthesis of type I porphyrins and of protoporphyrin III. The type I porphyrins are believed to be by-products of hemoglobin synthesis, and the type III porphyrins are regarded as intermediates in hemoglobin metabolism. Normally, the formation of type III porphyrins preponderates. Protoporphyrin III is present in erythrocytes, especially in the megaloblasts, erythroblasts, and reticulocytes. The protoporphyrin concentration of erythrocytes and bone marrow increases when the erythrocyte count falls below 40 per cent of the normal value. Coproporphyrin III can be formed from protoporphyrin III by the liver, and there is evidence that it is a product of the destruction of methemoglobin. Coproporphyrin I formation increases during exaggerated hematopoietic activity; in the condition known as

congenital porphyria, there is an independent synthesis of abnormal quantities of type I porphyrins.

Hemoglobin is quantitatively the most important iron-porphyrin complex of vertebrates; the circulating erythrocytes of an adult man contain about 800 gm. total hemoglobin, equivalent to 30 gm. of heme. The chief functions of hemoglobin are the transport of oxygen and carbon dioxide, and the maintenance of the pH of the blood. Hematopoietic tissues, which synthesize hemoglobin, include the embryonic blood islands, the fetal liver, spleen, bone marrow and lymph glands, and the adult red bone marrow in the flat bones and the epiphyses of long bones. Hemoglobin can be produced very rapidly in anemic animals. Absorbed radioactive iron salt is entirely converted to hemoglobin within one week. The synthesis of hemoglobin is regulated by the oxygen demand of tissues. At 14,000 to 15,000 feet above sea level, the reduced partial pressure of atmospheric oxygen causes an increase in the hemoglobin content of the blood of from 20 to 50 per cent. When chick embryos are asphyxiated, they develop into blood monsters with anarchistically enlarged blood vessels and anomalous sinuses that contain relatively enormous quantities of blood and hemoglobin. The myoglobin of muscles functions as a temporary storage mechanism for transported oxygen, which is liberated during muscular contraction. Myoglobin has a greater oxygen affinity than hemoglobin. Many tissues contain cytochrome oxidase, cytochromes, catalase and peroxidases in small quantities. These enzymes are low in fetal liver and hepatoma tissue. Hepatic and renal catalase activity decreases also during malignancy in other tissues. The nuclei of the hepatic cells contain cytochrome oxidase, but little catalase. Adrenalectomy lowers the cytochrome *c* and cytochrome oxidase content of the kidney and liver. The enzymatic activities of iron-porphyrin complexes have been considered on pages 97 to 100.

An adequate supply of ionizable iron salts is necessary for the biological synthesis of hemoglobin and other iron-porphyrin complexes. Although the iron of myoglobin and hematin cannot be used directly for hemoglobin synthesis, it has been reported that dietary hematin, bile pigments, and chlorophyll enhance the effectiveness of small doses of inorganic iron salts. The administration of iron in excess of the normal requirement does not stimulate an overproduction of hemoglobin. Small quantities of dietary copper salts are necessary for the biological synthesis of hemoglobin, cytochrome *a*, cytochrome oxidase, catalase, and the peroxidases. While copper is not a component of these porphyrin complexes, it is present in the phenol oxidases and cupreins of tissues (page 535), which evidently participate in the synthesis of porphine chromoproteins. The cytochrome oxidase activity of bone marrow is reduced markedly by copper deficiency, and it is increased by such hematopoietic stimuli as hemorrhage, anoxia, and cobalt administration. Details of copper and iron metabolism are given on pages 603 to 608.

Dietary protein is utilized in the synthesis of the heme and globin radicals of hemoglobin, and an adequate protein intake is necessary to prevent atrophy of the hematopoietic cells. Catabolic intermediates of protein metabolism are used for the same purpose when protein stores are depleted. A mixture of the ten essential amino acids is the most favorable protein material for hemoglobin synthesis. In hemorrhagic dogs, approximately 3 gm. of hemoglobin can be formed from each gram of injected plasma protein; intravenous injections of hemoglobin are quantitatively available for new hemoglobin production.

ERYTHROPOIESIS

Erythrocytes are formed in the hematopoietic tissues listed on page 541. The mother or stem cell is the hemocytoblast; in successive generations along the erythropoietic line, the hemoglobin content of the cytoplasm gradually increases, giving rise to megaloblasts. The latter mature into erythroblasts, which decrease in size to form normoblasts. Deficit of iron and copper salts interferes with the proper development of the normoblasts. The latter eventually lose their nuclei, and become erythrocytes. About 1 per cent of the circulating erythrocytes of the normal adult show reticular structures on supravital staining with brilliant cresyl blue; these cells are known as reticulocytes. The reticulocyte count increases during unusually rapid production of erythrocytes, as, for example, when the thermostable erythrocyte-maturing factor of liver is administered orally or parenterally to patients with pernicious anemia. This substance stimulates the maturation of megaloblasts to normoblasts, as well as the formation of leukocytes and blood platelets. The erythrocyte-maturing factor is produced in the normal gastrointestinal tract by the action of the enzymatic intrinsic factor on the dietary extrinsic factor. Normally, the erythrocyte-maturing factor is stored in the liver. Pterins (page 515) have been reported as producing a small reticulocyte response. Normal erythropoiesis requires adequate intake of iron and copper salts, erythrocyte-maturing factor (usually as the extrinsic precursor), vitamin B₆ (pyridoxin), and protein. It is stimulated by tissue anoxia, light (vitamin D), and the administration of ascorbic acid, protoporphyrin, thyroxine, xanthopterin, and excess cobalt salt. In castrated and hypophysectomized rats, androgens stimulate erythropoiesis while estrogens inhibit it.

Erythrocytic Indices

The circulating erythrocytes of man, and of most mammals, are plastic, biconcave, denucleated disks which consist largely of water, hemoglobin, and stroma. In amphibia, fishes, birds, and reptiles, the erythrocytes are larger elliptical or biconvex nucleated cells. Nucleated erythrocytes are also present in the circulation of the mammalian embryo, and in the

adult following hemorrhage. The average human erythrocyte has a diameter of $7.8\ \mu$, and a thickness of $2\ \mu$. The average erythrocyte count, erythrocyte volume and hemoglobin content of human whole blood are given in Table 94. These values are used to calculate the indices in parts 2 and 3 of the table.

Human erythrocytes contain at least two kinds of hemoglobin, a fetal and an adult type, together with traces of methemoglobin. The fetal type of hemoglobin resembles myoglobin in its comparatively high affinity for oxygen; it constitutes about three fourths of the total hemoglobin of the blood of the newborn. The fetal hemoglobin is replaced entirely by the adult type by the seventh month of life. The erythrocyte count and hemoglobin level are maximal in the fetus, owing to the relative anoxia which occurs in intrauterine life. The erythrocytes of the newborn are comparatively large, and their hemoglobin concentration is higher than that of adult erythrocytes. The high erythrocyte count, erythrocyte volume, and hemoglobin concentration of the newborn decrease rapidly until the second year of life; subsequently they rise slowly and reach the adult values at approximately the fifteenth year (Table 94). A sex difference in hemoglobin content appears with adolescence. The hemoglobin content of an individual's blood can vary as much as from 20 to 30 per cent in the course of a day. The erythrocyte count and hemoglobin content of blood are decreased in anemias, and they are increased by residence at high altitudes, strenuous exercise, injection of adrenaline, partial asphyxia, transfusion, active secretion of digestive juices, dehydration, traumatic shock, and polycythemic diseases. The spleen is a storehouse of erythrocytes; by contracting, it can elevate the erythrocyte count during exercise, after hemorrhage, at high altitudes, and so forth. The colloidal properties, fragility, and sedimentation of erythrocytes have been discussed on pages 57 to 59.

OXYGEN TRANSPORT

The blood can dissolve physically only 0.3 volume per cent oxygen, but the oxygenation of hemoglobin allows the transport of 20 volumes per cent of the gas in combined form. As indicated in Table 94, the oxygen capacity of human blood can be conveniently estimated by multiplying the per cent hemoglobin concentration by 1.36 (the oxygen-carrying capacity of 1 gm. of hemoglobin). At sea level, the atmosphere contains 20.95 volumes per cent oxygen, equivalent to a partial pressure of 160 mm. mercury. Alveolar air normally contains about 14 volumes per cent, equivalent to a partial pressure of 105 ± 5 mm. mercury. The hemoglobin in the arterial blood of the human adult is normally saturated with oxygen to the extent of 95 ± 3 per cent. Hence, arterial blood contains 19 ± 2 volumes per cent oxygen, equivalent to a partial pressure of 90 ± 5 mm. mercury. The oxygen tension of tissues is only 0 to 25 mm.

TABLE 94

(1) AVERAGE ERYTHROCYTE COUNT, ERYTHROCYTE VOLUME, HEMOGLOBIN CONTENT, AND OXYGEN CAPACITY OF HUMAN BLOOD

	A ERYTHROCYTE COUNT Millions Per Cu. Mm.)	B PACKED CELLS (Vol. Per Cent)	C HEMOGLOBIN (Per Cent)	OXYGEN CAPACITY ¹ (Vol. Per Cent)
At birth	5.0 ± 0.5	48 ± 4	17 ± 2	28 ± 3
At 2 years	4.3 ± 0.5	36 ± 4	12 ± 2	16 ± 2
Adult ²	5.1 ± 0.7	44.5 ± 6	15 ± 2	20 ± 2
Males	5.4 ± 0.8	47 ± 7	16 ± 2	21 ± 2
Females	4.8 ± 0.6	42 ± 5	14 ± 2	19 ± 2

(2) AVERAGE ERYTHROCYTE INDICES

	CORPUSCULAR HEMOGLOBIN $(10 \times \frac{C}{A})$ ($\gamma\gamma$) ³	CORPUSCULAR VOLUME $(10 \times \frac{B}{A})$ (Cu. μ)	CORPUSCULAR HEMOGLO- BIN CONCENTRATION $(100 \times \frac{C}{B})$ (Per Cent)
At birth	34	96	35
At 2 years	28	84	33
Adult	29 ± 2	87 ± 5	34 ± 2
Males	30	87	34
Females	29	87	33

(3) COMMON CLINICAL INDICES⁴

$$\text{Volume index} = \frac{\text{vol. per cent of packed cells} + B}{\text{erythrocyte count} + 5,000,000}$$

$$\text{Color index} = \frac{\text{per cent hemoglobin} + C}{\text{erythrocyte count} + 5,000,000}$$

$$\text{Saturation index} = \frac{\text{per cent hemoglobin} + C}{\text{vol. per cent of packed cells} + B}$$

¹ Calculated by multiplying C × 1.36 (except in infants under seven months).² The adult figures apply after fifteen years of age.³ The symbol $\gamma\gamma$ indicates a micromicrogram or 1×10^{-12} gram.⁴ These indices give the volume, hemoglobin content and hemoglobin concentration of the average erythrocyte, relative to the normal adult values. Hence, the indices are normally 1.0 ± 0.1 .

mercury; at these low tensions, oxyhemoglobin is dissociated to hemoglobin and oxygen. The venous hemoglobin is 75 ± 10 per cent saturated with oxygen, and venous blood contains 15 ± 3 volumes per cent oxygen, equivalent to a partial pressure of 40 mm. mercury. It is evident that 4 ± 1 volumes per cent of oxygen are normally removed from the blood by the tissues. The arterial oxygen saturation of the human fetus is under 60 per cent, and hence much lower than that of the adult.

The reaction, $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$, occurs with extreme rapidity. Combination of hemoglobin with oxygen in the lung capillaries is accelerated by raising the oxygen tension, or by lowering the carbon dioxide tension. The low oxygen tension and high carbon dioxide tension in the capillaries of the general tissues assist the dissociation of oxyhemoglobin to oxygen and hemoglobin. The dissociation of oxyhemoglobin is accelerated by the slight fall in pH which is produced in the capillaries by the entrance of carbon dioxide and lactic acid from the tissues. This influence is small when compared with that exerted by the low oxygen tension, but it is important in severe exercise or when the arterial oxygen saturation of hemoglobin is low. After short, severe exercise, the hemoglobin of venous blood may be only 25 to 35 per cent saturated with oxygen. The oxygenation of hemoglobin is not a linear function of the oxygen tension; at an altitude of 14,000 feet, the partial pressure of alveolar oxygen is only 60 mm. mercury, but hemoglobin is still 85 per cent saturated with oxygen.

The dissociation of oxyhemoglobin in the capillaries is affected by the tissue activity, the rate and volume of the capillary blood flow, the oxygen capacity of the blood, and the degree of saturation of the arterial hemoglobin. Shallow breathing decreases the volume of tidal air, and causes inefficient oxygenation of hemoglobin. A similar result is produced by insufficiency of the pulmonary ventilating surface in respiratory diseases, or by diversion of venous blood from the pulmonary circulation due to congenital defects of the cardiac septum. The conversion of hemoglobin to carboxyhemoglobin or methemoglobin interferes with oxygen transport. Carbon monoxide decreases the dissociation of oxyhemoglobin.

A decreased rate of blood flow through tissue capillaries allows greater dissociation of oxyhemoglobin to hemoglobin. Vasodilatation and increased rate of blood flow usually accompany activity of organs; and hence, the venous blood coming from an active organ usually contains less reduced hemoglobin than the blood from a resting organ. When an arm is immersed in water at a temperature of 45°C. , vasodilatation occurs and the blood flow is accelerated; the venous hemoglobin of the limb may be 94 per cent saturated with oxygen. Opposite circulatory conditions prevail when a limb is cooled; a cyanotic coloration then appears, and the oxyhemoglobin may be completely reduced. Severe chilling causes a return of the pink coloration; the oxyhemoglobin is poorly dissociated, owing to inhibition of tissue oxidation by the low temperature. When the hemo-

globin content and oxygen capacity of the blood are seriously reduced, as in anemia, the circulation must be increased to allow normal transport of oxygen and carbon dioxide. Since the average circulation time is from twenty to twenty-three seconds, a human erythrocyte can circulate more than 100,000 times, and transport more than 250 times its weight of oxygen in one month.

The functions of hemoglobin in carbon dioxide transport have been outlined on pages 21 to 24. In contrast to the elaborate chemical provisions for transporting oxygen and carbon dioxide, the blood can dissolve only a very small fraction of the nitrogen of inspired air. At high pressures (as in a diving bell) nitrogen becomes more soluble in the body fluids, and especially in the fatty tissues. Fats dissolve approximately five times as much nitrogen, argon, and oxygen as aqueous media, but less than twice as much helium. At high pressures, argon and nitrogen exert narcotic effects; these can be avoided in deep diving by the use of a helium-oxygen mixture. Sudden reduction of excessive pressure causes the formation of nitrogen gas bubbles in the tissues. When this occurs in the vascular network of the bone marrow, bones, central nervous system, and joints, it causes paralytic symptoms and pain (caisson disease). Gas embolism and the resulting pain and disability, known as "bends," can be prevented by gradual decompression with inhalation of oxygen.

CATABOLISM OF HEMOGLOBIN

Reticulo-endothelial cells engulf and digest effete or fragmented erythrocytes, and they form bile pigment from the hemoglobin. The inorganic iron (colloidal ferric hydroxide) which is produced during hemoglobin catabolism is the brown "hemosiderin" noted by the histologist in the reticulo-endothelial cells of the liver and spleen. If the circulating erythrocyte has an average life of approximately one hundred days, the daily hemoglobin catabolism in a man weighing 65 kg. would produce approximately 7.7 gm. globin, 280 mg. bile pigment, and 27 mg. iron. The protein and iron are retained within the body and utilized again, but the bile pigments are excretory metabolites. Experiments with N^{15} compounds show that turnover of the porphyrin and protein nitrogen of hemoglobin is slow within the erythrocytes.

Although protoporphyrin is believed to participate in the synthesis of hemoglobin, it does not accumulate during hemoglobin catabolism. The hemoglobin is first oxidized to the biliverdin-iron-globin complex, choleglobin (page 536). Combination of oxyhemoglobin, or hemoglobin with ascorbic acid or hydrogen peroxide is believed to be responsible for the formation of choleglobin. Approximately 1 per cent of the erythrocyte iron is normally present as choleglobin. This green bile pigment chromoprotein occurs in largest concentrations in erythrocytes which have low osmotic resistance. Choleglobin is fragmented to globin, iron hydroxide,

and biliverdin; traces of the latter are found in normal erythrocytes. Biliverdin is reduced to bilirubin in all tissues, except the skin. These catabolic reactions can occur in almost any portion of the body, as evidenced by the formation of "black and blue spots" of bile pigment from extravasated blood. Under ordinary circumstances, the hepatic Kupffer cells probably produce appreciable quantities of bile pigment. The body cannot use the bile pigments for the synthesis of new hemoglobin; their excretion is, therefore, an index to the catabolism of hemoglobin. About 30 per cent of the bile pigments may be derived from myoglobin catabolism.

Bilirubin is transported in the blood as the indirect reacting serum albumin-bilirubin complex, hemobilirubin. The plasma normally contains 0.5 ± 0.3 mg. per cent hemobilirubin; the blood bilirubin level is low after meals. The biliverdin content of normal human blood is 0.12 ± 0.08 mg. per cent. Bile pigment is usually absent from normal cerebrospinal fluid. Plasma hemobilirubin is increased by abnormal hematopoiesis, hemolysis, starvation, and by accelerated physiological destruction of hemoglobin (in late fetal life, and in the first few weeks of infant life).

EXCRETION OF BILE PIGMENTS

The hepatic polygonal cells normally excrete bilirubin and other bile pigments. The fetal liver starts to excrete pigments in the seventh month; the bile pigments gradually accumulate in the green meconium. Bilirubin (direct reacting) is the principal pigment of human bile. In the polygonal cells, the indirect reacting hemobilirubin complex is converted into direct reacting cholebilirubin, and the bilirubin is dissociated from the serum albumin complex, by the action of bile salts. When the liver is removed, hemobilirubin is no longer converted into cholebilirubin. The latter is much less diffusible than hemobilirubin, and when the biliary passage is occluded, the cholebilirubin formed by the polygonal cells accumulates in the blood plasma. When the plasma level exceeds 1.6 mg. per cent, cholebilirubin accumulates in the tissues (icterus or jaundice) and is excreted in the urine in appreciable quantities (bilirubinuria). Bilirubin does not pass the placenta readily. The plasma level required for the production of jaundice and bilirubinuria is higher in the case of hemobilirubin (as exemplified by hemolytic jaundice). There is no true threshold for bilirubin, inasmuch as normal urine contains about 0.3 mg. per cent of the pigment. Biliary excretion of bilirubin is increased when hemoglobin catabolism is accelerated. The intravenous injection of 1 mg. bilirubin per kg. of body weight (as sodium bilirubinate), in normal adults, results in the biliary excretion of 95 per cent of the injected pigment within four hours. The clinical applications of this liver function test are mentioned on pages 165 and 559. Persistent absence of bile pigments from the feces, and the excretion of clay colored, bulky, fatty stools indicate complete biliary obstruction.

At times, the excreted bilirubin is reoxidized to green biliverdin in the gastro-intestinal tract; this occurs readily in infants, and during diarrhea in adults. Normally, intestinal bacteria reduce bilirubin to the bilien, urobilin, and the colorless bilan, urobilinogen. About 2 mg. urobilinogen are excreted daily in normal urine, but the less saturated bilan, mesobilinogen, is found only in pathological urine. The urinary urobilinogen represents a fraction of the pigment which has been reabsorbed from the intestinal tract. When excretion of bile into the intestine is prevented, the urinary urobilinogen disappears. The daily fecal output of total urobilin (urobilin plus urobilinogen) is 200 ± 100 mg. for the normal adult. Urobilin is the principal fecal pigment; the urobilinogen of freshly collected feces is rapidly oxidized to urobilin when exposed to air. The total urobilin elimination is an index to hemoglobin catabolism.

Hemoglobin is not a normal excretory product; only when excessive hemolysis occurs and hemoglobin is present in the plasma in sufficient quantities is the chromoprotein excreted in the urine. Hemoglobinuria develops when the plasma hemoglobin level is above 135 mg. per cent. The pigment is excreted entirely by the glomeruli; the maximal tubular reabsorption (T_m) is 2.5 mg. per minute in dogs.

The urobilin compound, urochrome, is the chief pigment of normal urine. An adult excretes about 70 mg. urochrome daily. Urochrome is an endogenous metabolite, whose output is proportional to the basal metabolic rate. Urochrome excretion is increased by fever, obstructive jaundice, and by administration of thyroxine or acid; it is lowered in cachexia, senility, and renal disease, also by thyroidectomy and by the administration of alkali. Urochrome excretion is not affected by the protein or chlorophyll intake, or by hematopoietic and hemolytic processes.

EXCRETION OF PORPHYRINS

The porphyrins are comparatively stable substances which are slowly destroyed by the tissues. They tend to accumulate in the growing zones of bone, and they are excreted in the urine and feces. Small quantities of coproporphyrin I are normally excreted in the bile. The total porphyrin excretion of the normal human adult is about 350 γ per day. Most of this is found in the feces; from 20 to 120 γ are excreted daily in the urine. The normal fecal and urinary porphyrin consists chiefly of coproporphyrin I, together with traces of coproporphyrin III, protoporphyrin III and uroporphyrin I. The type I porphyrins are related to side reactions of porphine metabolism, the type III porphyrins to hemoglobin synthesis and, at times, to abnormal hemoglobin catabolism.

The fox squirrel exhibits a physiological porphyria, characterized by the deposition of uroporphyrin I in the bones and teeth, and the excretion of abnormal quantities of this porphyrin. A similar deep red uroporphyrin pigmentation of bone is observed in human congenital porphyrias. Con-

siderable uroporphyrin I and coproporphyrin III are found in the urine and feces of porphyric patients. When the urinary porphyrin concentration is high, it imparts a red coloration to the urine.

PATHOLOGY OF HEMOGLOBIN METABOLISM

*"All seems infected that the infected spy,
As all looks yellow to the jaundiced eye."*

— ALEXANDER POPE

HYPERHEMOGLOBINEMIA; POLYCYTHEMIAS

The hemoglobin content and erythrocyte count of the blood are elevated by increased oxygen demand (strenuous exercise, hyperthyroidism), partial asphyxia (in the normal fetus and newborn infant, certain cardiac diseases, respiratory obstructions, decreased oxygen tension of alveolar air at high altitudes), and dehydration or loss of plasma (burns, traumatic shock, etc.). An experimental form of polycythemia, with an increased reticulocyte count, can be produced in animals by the administration of cobalt salts. This form of polycythemia, and the polycythemias produced by administration of ephedrine, strenuous exercise, and low atmospheric pressure, can be alleviated by the administration of sodium nitrite, choline, or raw liver. Nitrite and choline may diminish polycythemia by causing vasodilatation and an increased oxygen supply in bone marrow, since their effects are counteracted by atropine.

Polycythemia vera differs from most of the symptomatic polycythemias cited above, in that it is accompanied by a markedly increased blood volume and is not secondary to systemic anoxia. However, it has been claimed that there is a decreased circulation to the bone marrow which causes local anoxia and stimulates overproduction of erythrocytes. In polycythemia vera, the erythrocyte count varies from 6 to 11 millions per cu. mm., the color index and sedimentation rate are low, and the basal metabolic rate is high. The excessive catabolism of hemoglobin causes hyperbilirubinemia and an increased urobilinogen excretion. The high viscosity of the blood and the decreased rate of circulation lead to capillary congestion, cyanosis, and a tendency toward thrombosis. Choline and raw liver therapy is of little value in polycythemia vera.

HYPHEMOGLOBINEMIA; ANEMIAS

Anemia may be defined as an abnormal lowering of blood hemoglobin concentration (usually below 80 per cent of the normal value). Anemias are caused by blood loss, increased destruction of erythrocytes, or decreased erythropoiesis. They are classified as *macrocytic*, *normocytic*, and *microcytic* anemias, on the basis of the corpuscular volume or the volume index

(Table 94, page 544). The decreased oxygen capacity of the blood, which accompanies anemia, is compensated by increased cardiac output and adaptation of the tissues to decreased oxygen tension. The arteriovenous oxygen difference, therefore, tends to remain normal in early stages of anemia. Frequently, the plasma protein and choline esterase concentrations are lowered, and the blood non-protein nitrogen is increased. Severe anemia gives rise to anoxic symptoms (page 555); a 6 per cent hemoglobin concentration (about 40 per cent of normal) is sufficient to permit adequate oxygen transport in the resting patient.

Acute hemorrhage causes a reduction in blood volume, and only when this symptom is corrected do the erythrocyte count and hemoglobin concentration fall. The regeneration of erythrocytes reaches a peak in about one week; it is accompanied by an increased reticulocyte count. Elevation of the hemoglobin content lags behind the erythrocyte count; it depends somewhat on the quantity of iron available. The plasma iron concentration falls (owing to accelerated hemoglobin production), and the plasma lipides tend to increase in this type of anemia. In *chronic hemorrhage*, the iron supply is more seriously depleted by the hyperplastic bone marrow. The volume, color, and saturation indices are low (microcytic hypochromic anemia). The erythrocytes show diminished fragility; hypobilirubinemia results from the diminished hemoglobin catabolism; and the blood lipide concentration is usually low.

Hemolytic anemias are characterized by increased destruction of erythrocytes. They result from such infections as malaria, *Clostr. welchii*, and streptococci; from aniline, chlorate, iodoacetate, lead, nitrobenzene, phenylhydrazine, saponin, or sulfonamide intoxication; and from unknown hemolytic agents in chronic hemolytic jaundice, the acute anemia of Lederer, sickle cell anemia, paroxysmal hemoglobinuria, and favism. Hemolytic anemias are usually characterized by increased bone marrow activity, moderate increase in plasma hemobilirubin concentration, and an increased excretion of urobilinogen. Severe acute hemolysis causes hemoglobinuria. In the anemias caused by arsenical or lead poisoning, there is increased excretion of coproporphyrin III. *Lederer's anemia* is accompanied by leukocytosis, reticulocytosis, and marked enlargement of the liver, spleen, and lymph nodes. *Chronic hemolytic jaundice* can be inherited as a dominant mendelian characteristic, or it can result from malaria (blackwater fever), syphilis, and other infections. In this type of jaundice, the erythrocytes show increased fragility associated with spherocytosis, whereas in obstructive jaundice the erythrocyte fragility is usually decreased. The congenital form of chronic hemolytic jaundice is accompanied by marked reticulocytosis. The ether insoluble fraction of deproteinized normal plasma contains a substance which inhibits the hemolysis. Hence, transfusions of plasma afford temporary relief; splenectomy often decreases the erythrocyte fragility, and leads to permanent improvement. *Sickle cell anemia* occurs almost exclusively in negroes;

the peculiarly shaped erythrocytes show increased fragility. The sickle forms tend to appear at low blood oxygen tensions, and revert to the normal shape in the presence of oxygen. Hemolytic anemia accompanies *paroxysmal hemoglobinuria* (page 560), one form of which occurs in certain syphilitic patients following exposure to cold. The blood of these patients exhibits the Donath-Landsteiner reaction *in vitro* (hemolysis after chilling the erythrocytes, mixing them with plasma, and warming the mixture to body temperature). In the *nocturnal type* of paroxysmal hemoglobinuria, the blood does not show the Donath-Landsteiner phenomenon; but the erythrocytes are exceptionally susceptible to a thermolabile hemolysin of normal plasma. Liver enlargement, hemosiderosis of the kidneys, and hepatic thrombosis and necrosis are common in this condition. *Favism* is an anaphylactic type of hemolytic anemia, caused by hypersensitivity to the fava bean. Jaundice, hemoglobinuria, and vascular symptoms are prominent in favism.

In the interesting congenital hemolytic anemia of infancy which is known as *erythroblastosis fetalis*, the fragility of erythrocytes is increased. The condition is associated with absence of Rh blood group factors in the mother, and their presence in fetal blood. The Rh agglutinogens are inherited as dominant mendelian characteristics, and they are found in the erythrocytes of approximately 85 per cent of human beings, and in practically all full-blooded American Indians. Intrauterine hemolysis of the fetal blood is caused by anti-Rh agglutinin, which appears first in the plasma of the Rh negative mother as the result of iso-immunization. Erythroblastosis usually occurs in the second or later pregnancy. The Rh isoagglutinins can cause stillbirth or late abortion, and occasionally they give rise to transfusion accidents in Rh negative patients.

Anemias characterized by deficient erythropoiesis and hemoglobin production include the hyperchromic macrocytic type caused by deficit of the erythrocyte-maturing factor or of vitamin B₁₂ (folic acid), the hypochromic microcytic type, caused by iron and pyridoxin deficiencies, and the variable types which accompany hypothyroidism and ascorbic acid deficiency.

Macrocytic Anemias

Typical *pernicious anemia* is the result of an inherited predisposition, which also leads to glossitis, atrophy of the gastro-intestinal mucosae, and sclerosis and demyelination of the central nervous system. Pernicious anemia patients exhibit complete dysfunction of the gastric glands, that is, achlorhydria and absence of gastric enzymes. Deficiency of the intrinsic factor interferes with the formation of an adequate quantity of the erythrocyte-maturing factor in the gastro-intestinal tract, and with its storage in the liver. During relapse of the disease, macrocytic hyperchromic anemia appears, with high volume and color indices and an essentially normal saturation index. Granulocytopenia and thrombocytopenia are present;

the blood volume is low; the plasma volume is increased. The increased plasma concentrations of hemobilirubin and iron, and the enhanced excretion of urobilinogen and coproporphyrin I, indicate active hemolysis. The cholesterol ester and phospholipide concentrations of the plasma are low, while the neutral fat is increased; the free cholesterol and phospholipide of the erythrocytes are low. The hypoprothrombinemia which occurs during relapse is not affected by vitamin K, but it responds to therapy with liver extract. During remission, whether spontaneous or due to the administration of the erythrocyte-maturing factor, the reticulocyte count rises and the erythrocytic indices return to normal. The reticulocyte count increases rapidly during the third to eighth days of liver therapy; subsequently, it diminishes again, while the hemoglobin concentration continues to rise for several weeks. The low blood cholesterol rises with the reticulocyte count; the plasma iron falls, and the excretion of urobilinogen and coproporphyrin I decreases. The achlorhydria is not remedied by liver therapy.

Gastro-intestinal diseases which are characterized by atrophy of the gastric mucosa, or by prolonged diarrhea, lead to deficient formation or absorption of the erythrocyte-maturing factor and a consequent macrocytic anemia. When the liver is damaged extensively, as in hepatic cirrhosis or malignancy, macrocytic anemia develops as the result of deficient storage or utilization of the erythrocyte-maturing factor. However, the kidney can store small quantities of this factor, and the anemia tends to be rather mild. These types of macrocytic anemia generally exhibit neither hyperbilirubinemia nor increased urobilinogen output. In sprue, pellagra, chronic alcoholism, gastro-colic fistulae, ileitis, intestinal stricture, and myxedema, macrocytic anemia can occur as the result of deficient intake of the extrinsic factor or deficient absorption of the erythrocyte-maturing factor. The achlorhydria and diarrhea which accompany these conditions are frequently corrected by liver therapy. Some of the patients develop microcytic anemia as the result of iron deficiency. The early fetus displays a macrocytic, hyperchromic anemia, which is probably the result of relative deficiency of the erythrocyte-maturing factor.

Microcytic Anemias

Hypochromic anemia is usually due to specific deficiency of iron, or of secondary factors concerned in iron utilization (copper salts, pyridoxin, and an undetermined liver substance). In this type of anemia the bone marrow activity is increased but the cells are microcytic, and have low volume, color and saturation indices. The plasma iron and bilirubin concentrations are low, and the hemoglobin catabolism and urobilinogen excretion decrease. Hypochromic anemia often develops in infants, adolescents, and women, associated with the high dietary iron requirements of growth, menstruation, and repeated pregnancies. The physiological

hypochromic anemia of infancy develops rapidly during the first six weeks of life; it is not relieved readily by the administration of iron. Later, a true iron deficiency anemia appears, if there is a low iron reserve or inadequate iron intake. While iron deficiency of the mother does not cause congenital anemia in the offspring, it does result in a low iron reserve. Premature and underweight infants have especially small reserves. Prolonged intake of an exclusive diet of cow's milk, and the occurrence of such gastro-intestinal complications as diarrhea, hypochlorhydria, infection, and malnutrition, hinder iron absorption in the young child. Iron-deficiency anemia of adolescent girls, termed *chlorosis* from the yellow-green complexion, is no longer a common disease. Achlorhydria, diarrhea, restricted diets, and chronic blood loss are important predisposing factors to hypochromic anemias in adults. Gastro-intestinal diseases, myxedema, scurvy, and deficit of pyridoxin (vitamin B₆) can cause hypochromic anemia.

Chronic benzene or arsphenamine poisoning causes *depression of bone marrow activity*, represses the formation of erythrocytes, leukocytes, and thrombocytes, and produces severe anemia and hemorrhage from mucous surfaces. Similar anemias result from the action of bacterial toxins in chronic infections, and from extensive destruction of bone marrow in leukemia, myeloma, lipidoses, and metastases to bone marrow. These anemias are usually hypochromic in type. The anemia which accompanies nitrogen retention in glomerulonephritis, nephrosclerosis, and other renal diseases is caused by depression of bone marrow activity; it is aggravated by dietary restriction. Radium, and other radioactive poisons, which are deposited in bones, inactivate the bone marrow and produce megalocytic anemia. The *aplastic anemia* which results from aplasia of bone marrow is characterized by greatly reduced erythrocyte, leukocyte, and thrombocyte counts, and by increased excretion of coproporphyrin III. The color index tends to be normal, and the plasma iron concentration is high. *Achrestic anemia* is a rare aplastic type of hyperchromic, megalocytic anemia. In this condition, the gastric juice is apparently normal and the bone marrow is hyperplastic, but an unexplained abnormality of the bone marrow prevents the utilization of liver extract.

Treatment of Anemias

In macrocytic anemias (including pernicious anemia), it is important to provide adequate quantities of erythrocyte-maturing factor, vitamins B₁₂ (thiamin) and B₂ (riboflavin), and folic acid. The curative effect of liver extract continues as long as it is administered, whereas the untreated disease progresses, with occasional remissions, to a fatal termination. Liver extract (or stomach extract) can be injected, or administered orally, to provide the erythrocyte-maturing factor. The latter is sixty times as effective parenterally as when given by mouth. Iron alone has little therapeutic value. The diet should contain liberal quantities of meat, liver, and

the other foods with high hematopoietic efficiency (Table 93, page 539). In other types of anemia, these foods are beneficial because of their iron, copper, and vitamin contents. The ingestion of moderate quantities of inorganic iron salts (preferably in the form of non-irritating ferric or ferrous salts) alleviates microcytic hypochromic anemia; it does not stimulate hemoglobin production in normal persons, or in patients with leukemia, chronic infections, or pernicious anemia. The oral administration of hydrochloric acid assists iron absorption when achlorhydria is present (page 603). Aplastic anemia does not respond to iron, or to liver therapy. Most of the amino acids necessary for globin synthesis can be obtained from endogenous protein catabolism but exogenous lysine appears to be necessary, since rats develop anemia when maintained on deaminized casein as the sole source of dietary protein. It is important to maintain the protein intake of anemia patients at a moderately high level in order to prevent weight loss and protein depletion. The protein intake is especially important in the anemias of pregnancy. Transfusions of whole blood (which has been preserved less than four days) afford relief from the anoxic symptoms; Lederer's anemia responds dramatically to this therapy. Plasma transfusions are of temporary value in congenital hemolytic jaundice and in sickle cell anemia. The administration of liver and of autoclaved yeast aids in the cure of the anemias of pellagra, sprue and of tropical macrocytic anemia. The active liver principle which alleviates the latter condition differs from the extrinsic and the erythrocyte-maturing factors. Certain liver fractions, folic acid, and pyridoxin combat the anemia and agranulocytosis caused by sulfonamides. Pyridoxin can prevent anemia from typhus toxin in rabbits. Ascorbic acid, pyridoxin, and ultraviolet light (vitamin D) stimulate erythropoiesis in certain microcytic hypochromic anemias of childhood. The anemia of scurvy is uninfluenced by iron or liver extract, unless ascorbic acid is also provided; and in the anemia of myxedema, the thyroid deficiency must be corrected by the administration of thyroid to render iron and liver therapy effective.

ANOXIA

This term is used to designate oxygen lack and retarded aerobic oxidation in tissues. Complete anoxia causes death within a few minutes, although certain isolated tissues can withstand anoxia for several hours. *Anoxic anoxia (anoxemia)* is caused by decreased oxygen content of the arterial blood; when the condition is the result of anemia, carbon monoxide poisoning, methemoglobinemia, or sulfhemoglobinemia it is termed *anemic anoxia*. Anoxic anoxia occurs at high altitudes, and in respiratory and cardiac diseases (asthma, bronchitis, emphysema, pneumonia, pulmonary edema, pulmonary exudate, tuberculosis, uncompensated cardiac disease, and cardiac septal defects). When the circulation to the patho-

logical portion of a lung is occluded, as in early lobar pneumonia, pneumothorax, or pleural effusion, the anoxia disappears. At least half of the lung area can be obliterated without significant lowering of the oxygen saturation of arterial blood, provided the circulation is shifted to the unaffected pulmonary area. Retardation of the circulation in shock, cardiac failure, or contraction of the arterioles (Raynaud's disease), causes *stagnant anoxia*; and poisoning of the cellular oxidases and dehydrogenases by cyanides or narcotics gives rise to *histotoxic anoxia*. In these two types of anoxia, the arterial oxygen tension can be normal. In stagnant anoxia, the venous blood is abnormally unsaturated, but in histotoxic anoxia it is near the normal state of saturation. Cyanide combines with methemoglobin and cytochrome oxidase more readily than with hemoglobin or oxyhemoglobin, and it causes very rapid anoxia.

Anoxia is an important practical problem of aviation. Symptoms usually appear at altitudes of 6,000 to 10,000 feet; at the danger zone (13,000 to 16,000 feet) arterial oxygen saturation is only 80 per cent. Convulsions, coma, and death may be expected at altitudes above 20,000 feet. Hence, inhalation of oxygen is recommended above 8,000 feet, and it is practiced routinely above 15,000 feet. When an altitude of 30,000 feet is reached, the gas must be administered under pressure. At 50,000 feet, even 100 per cent oxygen will not penetrate the alveoli unless it is under pressure, since the atmospheric pressure at this level (86 mm. of mercury) does not exceed the sum of the alveolar tensions of water vapor and carbon dioxide (47 and 39 mm. of mercury, respectively).

Nerve function ceases a few seconds after sudden, complete oxygen withdrawal; but the tissues can adapt their function and oxygen consumption to a gradually decreasing oxygen tension. Sudden or acute anoxia gives little warning; progressive numbness appears, first in the extremities, and the patient becomes unconscious. Under these circumstances, the central nervous effects precede the cardiac symptoms. Cerebral edema develops, and coma of cerebral origin precedes the cessation of respiration. Respiratory paralysis occurs early in the histotoxic anoxias. The symptoms of a gradually developing anoxia are preponderantly cardiac in origin, and they are referable to circulatory failure. They include weakness, dizziness, stupor, syncope, numbness, tingling, headache, hallucinations, and disturbances of special senses. The pulse and respiration are accelerated, and dyspnea occurs on exertion. Cyanosis is present, except in the anemic and histotoxic anoxias. When the arterial saturation is less than 60 per cent, the respiratory and circulatory mechanisms become less efficient. The hyperpnea of compensated anoxia causes respiratory alkalosis; otherwise, anoxia causes acidosis.

Morphine tends to increase anoxia by slowing the respiration. Oxygen inhalation is important for the relief of all types of anoxia, except that due to cyanide poisoning. Prolonged breathing of pure oxygen at atmospheric

pressure can induce pulmonary irritation; at higher pressures it causes nausea, vasoconstriction, and convulsions. Digitalis aids in alleviating the anoxia resulting from decompensated cardiac disease.

CYANOSIS

This condition is characterized by a diffuse lilac-blue to brown-blue coloration of the skin; it is due to abnormal concentrations of dark colored chromoproteins in the capillary blood. Hemoglobin is usually the responsible agent, and less frequently methemoglobin or sulfhemoglobin is concerned. Cyanosis requires the presence of at least 5 gm. of reduced hemoglobin per 100 ml. of capillary blood; at a normal hemoglobin concentration, this corresponds to an oxygen saturation of 67 per cent (85 per cent in the arterial blood). Anemia patients whose total hemoglobin concentration is less than 5 per cent cannot become cyanotic, but polycythemic patients develop cyanosis readily. The threshold value is affected by the thickness and pigmentation of the skin, the presence of lipemia, leukemia, and carotenemia, and variations in the capillary bed. Cyanosis results from circulatory stasis, and from all types of anoxia except the histotoxic and severe anemic forms. Local cyanosis occurs in the extremities as the result of decreased blood flow, or shunting of the circulation. In the affected tissues, the dissociation of oxyhemoglobin is increased, thus supplying the oxygen required by the tissues. Cyanosis is most closely related to arterial unsaturation; anoxic symptoms, to venous unsaturation; and stagnation or venous congestion, to the arteriovenous oxygen difference.

CARBON MONOXIDE POISONING

The bright red color of carboxylhemoglobin is visible in the lips and cheeks of persons poisoned with carbon monoxide. The affinity of hemoglobin for carbon monoxide is two hundred and ten times its affinity for oxygen. Carbon monoxide poisoning, therefore, diminishes the oxygen-carrying capacity of the blood and interferes with the oxygen supply of the tissues. Very high carbon monoxide concentrations (about a thousand times that which will asphyxiate a man) are necessary to inhibit the cytochrome oxidase in tissues. The anoxia of carbon monoxide poisoning is particularly severe because carboxylhemoglobin interferes with the dissociation of the remaining oxyhemoglobin. One-half saturation of the blood with carbon monoxide occurs at atmospheric concentrations of 0.1 per cent, and causes severe, acute, anoxic symptoms. Breathing air which contains more than 0.02 per cent of carbon monoxide eventually causes toxic effects. As in other anoxias, the brain is very susceptible to edema and to fatal degenerative changes if the intoxication is prolonged. Hence, resuscitation should be performed speedily by administering a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, which

accelerates respiration, saturates the blood with oxygen, and gradually displaces the carbon monoxide. The inspired carbon dioxide lowers the blood pH, and thus assists in the dissociation of the carbonylhemoglobin. The carbon monoxide can be displaced entirely within a few hours; but the occurrence of cerebral damage may lead to a fatal termination within several days, or to permanent injury (paralysis agitans).

METHEMOGLOBINEMIA AND CYANHEMOGLOBINEMIA

Traces of methemoglobin are formed normally by the oxidation of hemoglobin. After administration of the poisons mentioned on page 532, it accumulates in the blood stream. Acetanilide, antipyrine, phenacetin (acetophenetidin), sulfanilamide, and sulfapyridine are responsible for most clinical cases of methemoglobinemia. The condition is frequent among industrial workers who come in contact with coal tar products. Methemoglobinemia also occurs in certain infections and hemolytic diseases (blackwater fever, hemolytic jaundice, paroxysmal hemoglobinuria). Idiopathic methemoglobinemia is a rare congenital condition, which is not amenable to ordinary treatment. Methemoglobin reverts to hemoglobin rather slowly in the human body; the administration of moderate quantities of methylene blue accelerates this reduction. In rabbits, methemoglobin is destroyed rapidly; and the net result of methemoglobinemic poisons, in this animal, is the production of anemia. In methemoglobinemia, the blood hemoglobin (as usually determined) has a low oxygen capacity. Cyanosis and other anoxic symptoms result from methemoglobinemia; the cyanosis is intense, owing to the dark brown color of the methemoglobin. Transformation of two thirds of the blood hemoglobin to methemoglobin is fatal.

Cyanhemoglobin is formed by the interaction of cyanides and methemoglobin. This reaction is the basis of the nitrite therapy of cyanide poisoning. Sodium nitrite is administered to convert hemoglobin to methemoglobin, which unites with cyanide and assists in preventing its combination with the cytochrome oxidase of cells. The cyanhemoglobin is gradually converted to oxyhemoglobin and cyanate, which is not toxic. Injection of sodium thiosulfate accelerates the formation of cyanate. Large doses of methylene blue may be used in place of the nitrite, but the latter is much more effective in producing methemoglobinemia.

SULFHEMOGLOBINEMIA

The symptoms of this intoxication resemble those of methemoglobinemia. Sulfhemoglobin is produced by the combined action of hydrogen peroxide and a sulfide upon reduced hemoglobin. Since sulfhemoglobin is not reconverted to hemoglobin, it remains in the blood longer than does methemoglobin. Sulfhemoglobinemia occasionally results from the inges-

tion of aniline derivatives, particularly acetanilide, sulfanilamide, and sulfapyridine. It is not produced by the ingestion of sulfides, or by the inhalation of hydrogen sulfide; these substances are rapidly oxidized to sulfate *in vivo*.

HYPOBILIRUBINEMIA

Slight decreases in blood bilirubin occur in anemias characterized by decreased hemoglobin catabolism, as for example, posthemorrhagic and aplastic anemias, chlorosis, and the anemias of malignancy and chronic nephritis.

HYPERBILIRUBINEMIA AND JAUNDICE (ICTERUS)

Hyperbilirubinemia results from (a) obstruction of the bile flow, with accumulation of direct reacting cholebilirubin in the blood; and (b) excessive destruction of hemoglobin, with accumulation of indirect reacting hemobilirubin. The corresponding types of jaundice are known as obstructive and hemolytic icterus, respectively. Hemolytic jaundice is not dependent on hepatic or biliary pathology, although it is at times associated with it. Hepatogenous jaundice is the type which results from parenchymatous liver damage. Whenever the extrahepatic biliary passages are obstructed, there is some injury to the hepatic parenchyma, and vice versa, so that obstructive and hepatogenous types of jaundice represent gross clinical distinctions.

In obstructive jaundice, when the icteric index rises above 15 and the serum bilirubin (Van den Bergh method) exceeds 1.8 ± 0.2 mg. per cent, the yellow tissue pigmentation is visible in the skin, sclera, and mucous membranes. Between this threshold and the normal blood level (icteric index of 4 to 6, and 0.5 mg. per cent bilirubin), there is a zone of *latent jaundice*. Repeated bilirubin determinations are particularly useful for investigating hemolytic processes, hepatic function, and biliary physiology when jaundice is not visible, or when biliary surgery is contemplated. Carotenemia, which is especially prominent in diabetic patients, and in children on vegetable diets, falsifies the icteric index, unless the carotenoid pigments are first precipitated from the serum by the addition of two volumes of acetone. Xanthosis, or the skin coloration which accompanies carotenemia, can be mistaken for jaundice. Determination of the serum or plasma bilirubin concentration by the Van den Bergh reaction is more accurate than the icteric index; the Van den Bergh qualitative test also allows the differentiation of indirect reacting hemobilirubin from direct reacting cholebilirubin. The so-called biphasic or delayed reaction is interpreted as one type of the direct reaction. Since cholebilirubin diffuses into tissues more rapidly than does hemobilirubin, jaundice is more frequent and more pronounced in biliary obstruction than in hemolytic disease. Cholebilirubin has the lower threshold value for urinary excre-

tion. In obstructive jaundice, as the hyperbilirubinemia progresses, the Van den Bergh qualitative test changes from an indirect reaction, through a biphasic type, to an immediate direct reaction. Biliary calculi are the most common cause of obstructive jaundice; occasionally, the obstruction is due to parasitic infestations, pathology of the duct walls (atresia, cholangitis, stenosis, tumors), or compression of the common bile duct by adjacent tumors. In severe functional insufficiency of the hepatic polygonal cells, cholebilirubin is present in the blood although there is no obstruction of the biliary passages. In early non-obstructive hepatogenous jaundice, the hyperbilirubinemia is due to retention of hemobilirubin; later, the liver pathology causes retention of cholebilirubin. Infectious jaundice of this type occurs in acute hepatitis, catarrhal jaundice, spirochetal jaundice, yellow fever, etc.; so-called toxic hepatogenous jaundice results from poisoning by arsphenamine, carbon tetrachloride, chloroform, cinchophen, lead, mercury, and phenylhydrazine, and from acute yellow atrophy, congestive cardiac failure, diabetes, toxemias of pregnancy, and x-ray overdosage.

Hemolytic jaundice has been discussed on page 550. A similar accumulation of hemobilirubin, *icterus neonatorum*, results from the physiological hemolytic processes which occur during the first two weeks of life. Hyperhemobilirubinemia occurs in hemolytic streptococcus infections, malaria, oroya fever, pernicious anemia, paroxysmal hemoglobinuria, polycythemia, sickle cell anemia, sprue, and posttransfusion reactions. The anoxia, which accompanies anemic conditions and congestive cardiac failure, tends to provoke functional insufficiency of the polygonal cells and a moderate hyperbilirubinemia.

Xanthochromic is an accumulation of *hemobilirubin* in spinal fluid. It is present after hemorrhage into the subarachnoid space, when hemolysis occurs and the fluid becomes yellow. This coloration gradually disappears as the pigment is reabsorbed. In advanced jaundice, especially the obstructive type, bilirubin can enter the cerebrospinal fluid from the blood stream. A physiological xanthochromia accompanies *icterus neonatorum* of premature infants. In this condition, and in the xanthochromia of severe obstructive jaundice, the permeability of the cerebrospinal barrier is increased. Xanthochromia also accompanies intramedullary tumors, tumors of the cauda equinae, tuberculosis of the vertebrae, acute myelitis, and chronic meningitis. In the Froin syndrome, which accompanies spinal cord tumors, the lumbar xanthochromic fluid contains much protein and tends to coagulate spontaneously.

Bilirubin Excretion Test

In this test, 1 mg. bilirubin per kg. of body weight is injected intravenously; the serum bilirubin is then determined in blood samples taken immediately, at five minutes, and at four hours. In normal persons,

the bilirubin concentration of the blood at the fourth hour is less than 6 per cent of the difference between the concentrations in the first and second samples. Greater retention indicates hepatic impairment. The test is expensive, and it gives useful information only when the blood bilirubin level is 1 mg. per cent, or less.

Cirrhosis

A degenerative condition of the liver, termed cirrhosis, is frequently associated with jaundice. The common portal type of cirrhosis is usually preceded by fatty infiltration and accompanied by ascites (page 617). Biliary cirrhosis results from prolonged biliary obstruction, and pigment cirrhosis from pernicious anemia or hemochromatosis. Toxic cirrhosis is caused by alcohol, arsenical drugs, cinchophen, carbon tetrachloride, chloroform, bacterial toxins, and so forth, often in combination with dietary deficiencies. Experimental cirrhosis can be produced in rats by a low protein or low methionine intake. Addition of cystine or cysteine to such diets accelerates the development of cirrhosis, while methionine and choline prevent hepatic injury by toxic agents (page 235). Intravenous administration of the sodium salts of xanthine or uracil, and of ricinoleic acid, tends to protect livers against hepatotoxic agents. A high fat diet induces maximal susceptibility to hepatic damage, while sufficient carbohydrate and protein are protective. Dietary protein and essential amino acid mixtures stimulate the growth and regeneration of liver cells. Since meat extracts increase ascites, dairy proteins are substituted for meat in the diet of the cirrhosis patient. The optimal diet contains 75 per cent carbohydrate, 20 per cent protein, and 5 per cent fat. Injection of crude liver extract diminishes the ascites.

HEMOGLOBINURIA AND MYOGLOBINURIA

Urinary excretion of hemoglobin (as distinguished from hematuria, or the presence of erythrocytes in urine) indicates massive hemolysis. It occurs when more than 135 mg. per cent of free hemoglobin is present in the blood plasma, as in paroxysmal hemoglobinuria, blackwater fever of severe malaria (especially after the administration of quinine), severe hemolytic infections (hemolytic streptococcemia, scarlet fever, typhoid fever, yellow fever), transfusion of incompatible blood, severe intra-abdominal hemorrhage, severe burns, prolonged exposure to cold, and, at times, in hemolytic jaundice and in poisoning by arsphenamine, carbon monoxide, phosphorus, potassium chlorate, various benzene compounds, and ricin. Hemoglobinuria is frequently accompanied by chills, fever, vomiting, vascular spasms and urticaria.

There are several *paroxysmal types* of hemoglobinuria. Nocturnal paroxysmal hemoglobinuria occurs in chronic hemolytic anemia. The

paroxysmal hemoglobinuria of the syphilitic patient has been discussed on page 551. A benign form of paroxysmal hemoglobinuria, known as march hemoglobinuria, is the result of intravascular hemolysis in young men following energetic walking or running. The rare, fatal paralytic variety of hemoglobinuria is actually myoglobinuria; it results from crushing injuries or other muscular damage. The glomeruli excrete myoglobin rapidly, and deposition of this chromoprotein in the tubules can cause renal injury. Urinary myoglobin is detected spectrophotometrically (page 529). Red colorations appear in the urine, not only in the above conditions, but also during hematuria, porphyria, excretion of administered phenolphthalein, and after excessive ingestion of beets.

BILIRUBINURIA

When the serum cholebilirubin concentration exceeds 1.8 ± 0.2 mg. per cent, excessive quantities of the pigment are excreted in the urine. Uremic conditions tend to raise this threshold. The threshold for hemobilirubin excretion is higher; hence, bilirubinuria is more frequent in obstructive than in hemolytic jaundice.

UROBILINURIA AND FECAL UROBILIN EXCRETION

The fecal excretion of total urobilin (urobilin plus urobilinogen, normally 200 ± 100 mg. per cent) is increased in hemolytic jaundice, malarial chills, paroxysmal hemoglobinuria, polycythemia, and during relapse of pernicious anemia. The fecal urobilin excretion is decreased in gastrointestinal obstruction and in obstructive jaundice. Clay colored stools usually contain little urobilin, although at times the pigment is obscured by large quantities of unabsorbed fat. In such cases, extraction of the fecal fat, or duodenal intubation, will reveal the presence of the urobilin. In non-obstructive conditions, the total urobilinogen excretion reflects the rate of hemoglobin catabolism. Low total urobilin output in the feces and urine results from starvation, hypochromic anemias, leukemia, and macrocytic anemias other than pernicious anemia.

Moderate liver damage and incomplete biliary obstruction cause increased urinary excretion of urobilinogen, owing to deficient hepatic excretion of the urobilinogen reabsorbed from the intestine. Urobilinuria is, therefore, an index to hepatic insufficiency. When complete obstruction of the common bile duct develops, as in neoplasms, bile fistulae, cholangitis, cholelithiasis, and congenital atresia of the common bile duct, both urobilin and urobilinogen tend to disappear from the feces and urine, while the urinary bilirubin excretion continues. Urobilinogen does not always disappear from the urine under these circumstances, since bacteria in the biliary passages above the obstruction can produce urobilin, and a small quantity of bile pigment can enter the intestine through severely

jaundiced mucosa. Urobilinogen is formed occasionally in infarcted areas, hemorrhagic ovarian cysts, and hematomas. In the absence of such complications, urobilinuria indicates either functional impairment of the liver or active infection of the bile ducts. Urobilinuria is one of the most sensitive indices to liver dysfunction; it can occur without jaundice, in diffuse hepatic disease (toxic infections, early stages of portal cirrhosis). Urobilinuria precedes hyperbilirubinemia in congestive heart failure, cholelithiasis, and hepatitis. Estimation of the urinary urobilinogen is, therefore, valuable in preoperative studies. Because of the great variability in urobilinogen excretion, twenty-four hour specimens of urine should always be used for the determinations.

PORPHYRIA

Clinical syndromes which are characterized by abnormal excretion of porphyrins are termed porphyrias. In these diseases, the urine may contain from 300 to 50,000 γ of porphyrins daily, and large quantities are excreted in the feces. There are three clinical types of primary porphyria, namely, congenital, acute, and chronic porphyrias. Porphyrinuria can occur in aplastic anemia, fevers, xeroderma pigmentosa, chronic alcoholism, hemolytic diseases (pernicious anemia, hemolytic jaundice, etc.), hepatic diseases (cirrhosis, tumors, hemochromatosis, etc.), and as the result of poisoning by cinchophen, hypnotics (sulfonal, trional, veronal), lead, phosphorus, trinitrotoluene, arsphenamine and sulfonamides. Excretion of uroporphyrin I predominates in the congenital porphyrias, and of coproporphyrin I in the porphyrinurias accompanying hepatic and hemolytic diseases; while in other porphyrias and porphyrinurias, the chief urinary porphyrins are usually type III. It will be recalled that, under ordinary conditions, the coproporphyrin I excretion in feces and urine is an index to hematopoietic activity. The excretion of this pigment, and the fecal excretion of protoporphyrin III, are increased in hemolytic jaundice, hepatic disease, and in relapse of pernicious anemia. In the latter condition, the concentrations of both porphyrins increase in the bone marrow.

The chronic condition termed *acute porphyria* is inherited as a dominant mendelian characteristic. It is most common in women. The symptoms of acute porphyria include abdominal pain, vomiting, cardiovascular symptoms, symmetrical progressive paresis, and psychic disturbances. The urine may be pink, red, or deep brown; or it may have a normal color when the pigments are excreted as porphyrinogens. Coproporphyrin III is usually the chief urinary porphyrin in this condition. The porphyrinuria is most marked during the abdominal and paralytic attacks, which are frequently associated with menstruation. The feces of the patients contain uroporphyrin I and coproporphyrins I and III. The excessive production

of coproporphyrin III does not lead to deposition in the bones, or to light sensitivity.

The symptoms of the toxic porphyrias, in which coproporphyrin III is usually the major pigment, resemble those of acute porphyria. Porphyrinuria is diagnostic for lead poisoning. In acute lead poisoning, coproporphyrin III predominates in the urine, and coproporphyrin I in the feces. The protoporphyrin III content of the blood and bone marrow is increased, and also the coproporphyrin concentration of the bone marrow.

The *congenital* type of porphyria predominates in males. This condition is inherited as a recessive mendelian characteristic. It appears in early life, sometimes in the fetus, and it runs a long course. It is characterized by photosensitivity of the skin in spring and summer, blistering and scar formation, red-brown to purple pigmentation of the teeth and bones, mutilation from the severe skin lesions, and at times by hirsutism. The uroporphyrins tend to accumulate in the calcifying zones of bone. The skin lesions resemble those of hydroa aestivale and hydroa vacciniforme, but these conditions are known to occur independently of porphyria. In congenital porphyria, there is a disproportional synthesis of the type I porphyrins responsible for the photosensitizing effects. The ready combination of coproporphyrins with serum albumin, and with other proteins, inhibits their photosensitizing action. In white-furred animals, and in man, the injection of hematoporphyrin produces abnormal sensitivity to ultraviolet light; the skin becomes erythematous, edematous, and necrotic, and the experimental animals die when exposed sufficiently to intense light. The photosensitivity caused by hematoporphyrin is of long duration. Milder photosensitivity results from the injection of other porphyrins. In congenital porphyria, the urine and feces contain considerable uroporphyrin I and coproporphyrin I, and less of the type III porphyrins. The urine is usually red colored. When colorless porphyrinogens are excreted, exposure of the urine to light causes darkening. The porphyria is decreased by injection of ascorbic acid or liver extract. The bile and blood plasma contain coproporphyrin I; the bones and teeth uroporphyrin I; and the bone marrow, kidneys, and liver have both pigments. Congenital porphyria occurs in swine and cattle. Excessive quantities of protoporphyrin III have been reported in chloroma tumors, which accompany certain forms of leukemia.

In the *chronic* type of porphyria, there is some sensitivity to light, and gastro-intestinal symptoms are present. Both types of coproporphyrins and uroporphyrins are excreted, principally in the feces.

Other diseases caused by photosensitization occur occasionally in animals. Phylloerythrin, formed from chlorophyll in the gastro-intestinal tract of ruminants, is the photosensitizing factor in geeldikkop. Animals with this disease exhibit marked jaundice and edema of the skin. The

phylloerythrin is normally excreted in the bile, so that obstructive jaundice is a prerequisite to the development of photosensitization. The quantities of chlorophyll and buckwheat ingested by human beings are insufficient to induce the geeldikkop and fagopyrism observed in domestic animals.

BIBLIOGRAPHY

NUCLEIC ACIDS

Chemistry

- GILMAN, H. Organic Chemistry. Vol. II. New York, Wiley, 1938.
 GREENSTEIN, J. P. Nucleoproteins. *Adv. in Prot. Chem.*, 1 : 209, 1944.
 LEVENE, P. A. T., and BASS, L. W. Nucleic Acids. New York, Chemical Catalog Co., 1931.

Metabolism

- DAVIDSON, J. N., and WAYMOUTH, C. Nucleic acids and tissue growth. *Nutrition Abstr. & Rev.*, 14 : 1, 1944.
 DRURY, A. N. Physiological activity of nucleic acid and its derivatives. *Physiol. Rev.*, 16 : 292, 1936.
 LUTWAK-MANN, C. Adenine derivatives and their biological functions. *Biol. Rev. Cambridge Phil. Soc.*, 14 : 399, 1939.
 MIRSKY, A. E. Chromosomes and nucleoproteins. *Adv. in Enzymol.*, 3 : 1, 1943.

Pathology

- BRÖCHNER-MORTENSEN, K. Uric acid in blood and urine in health and disease. *Medicine*, 19 : 161, 1940.
 COMROE, B. I. Arthritis and Allied Conditions. Ed. 3. Philadelphia, Lea and Febiger, 1944.
 TALBOTT, J. H. Gout. New York, Oxford Univ. Press, 1943.

PORPHYRINS AND RELATED PROSTHETIC PIGMENTS

Chemistry

- GILMAN, H. Organic Chemistry. Ed. 2. Vol. II. New York, Wiley, 1943.
 LEDERER, E. Pigments of the invertebrates. *Biol. Rev. Cambridge Phil. Soc.*, 15 : 273, 1940.
 MAYER, F. The Chemistry of Natural Coloring Matters. New York, Reinhold, 1943.
 MILLER, E. S. Quantitative Biological Spectroscopy. Minneapolis, Burgess, 1940. (Methods.)
 PAULING, L. C. The Nature of the Chemical Bond and the Structure of Molecules and Crystals. Ed. 2. Ithaca, Cornell Univ. Press, 1940.
 REDFIELD, A. C. The hemocyanins. *Biol. Rev. Cambridge Phil. Soc.*, 9 : 175, 1934.
 WATSON, C. J. The bile pigments. *New England J. Med.*, 227 : 665, 1942.
 ZSCHEILE, F. P. Plastid pigments. *Botan. Rev.*, 7 : 587, 1941.

Tissue Porphyrins

- MILLIKAN, G. A. Muscle hemoglobin. *Physiol. Rev.*, 19 : 503, 1939.
WHIPPLE, G. H. Hemoglobin and plasma proteins: their production, utilization and interrelation. *Am. J. M. Sc.*, 203 : 477, 1942.
WHIPPLE, G. H., and MADDEN, S. C. Hemoglobin, plasma protein and cell protein. *Medicine*, 23 : 215, 1944.

Erythropoiesis

- DOWNEY, H. Handbook of Hematology. New York, Hoeber, 1939. (4 vol.)
WAKERLIN, G. E. The hematopoietic liver principle. *Ann. Int. Med.*, 11 : 31, 1937.

Erythrocytic Indices

- DOWNEY, H. Handbook of Hematology. New York, Hoeber, 1939. (4 vol.)
HADEN, R. L. Principles of Hematology. Philadelphia, Lea and Febiger, 1939.
OSGOOD, E. E. Textbook of Laboratory Diagnosis. Ed. 3. Philadelphia, Blakiston, 1940.

Metabolism of Bile Pigments

- SOBOTKA, H. Physiological Chemistry of Bile. Baltimore, Wm. Wood, 1937.

Metabolism of Porphyrins

- BRUGSCH, J. T. Man and chlorophyll. *Ergebn. inn. Med. Kinderk.*, 56 : 614, 1939.
DOBRINER, K., and RHOADS, C. P. The porphyrins in health and disease. *Physiol. Rev.*, 20 : 416, 1940.
WELCKER, M. L. The porphyrins. *New England J. Med.*, 232 : 11, 1945.

PATHOLOGY OF HEMOGLOBIN METABOLISM

General

- DOWNEY, H. Handbook of Hematology. New York, Hoeber, 1938. (4 vol.)
KILDUFFE, R. A., and DEBAKEY, M. The Blood Bank and the Technique and Therapeutics of Transfusions. St. Louis, Mosby, 1942.
KRACKE, R. R., and GARVER, H. E. Diseases of the Blood and Atlas of Hematology. Ed. 2. Philadelphia, Lippincott, 1941.
KUGELMASS, I. N. Blood Disorders in Children. New York, Oxford Univ. Press, 1941.
WHITBY, L. E. H., and BRITTON, C. J. C. Disorders of the Blood. Ed. 3. Philadelphia, Blakiston, 1939.
WIENER, A. S. Blood Groups and Blood Transfusion. Ed. 3. Springfield, Thomas, 1943.
WINTROBE, M. M. Clinical Hematology. Philadelphia, Lea and Febiger, 1942.

Polycythemia

- HARROP, G. A. Polycythemia. *Medicine*, 7 : 291, 1928.

Anemias

CASTLE, W. B., and MINOT, G. R. *Pathological Physiology and Clinical Description of the Anemias*. London, Oxford Univ. Press, 1936.

Hemolytic Anemias; Hemoglobinuria

DAMESHEK, W., and SCHWARTZ, S. O. Acute hemolytic anemia. *Medicine*, 19 : 231, 1940.

GILLIGAN, D. R., and BLUMGART, H. L. March hemoglobinuria. *Medicine*, 20 : 341, 1941.

LUISADA, A. Favism. *Medicine*, 20 : 229, 1941.

YULE, C. L. Hemoglobinuria. *Physiol. Rev.*, 22 : 19, 1942.

Macrocytic Anemias; Pernicious Anemia

MINOT, G. R., and STRAUSS, M. B. Physiology of antipernicious anemia material. *Vitamins and Hormones*, 1 : 269, 1943.

MURPHY, W. P. *Anemia in Practice. Pernicious Anemia*. Philadelphia, Saunders, 1939.

WILLS, L., and EVANS, B. D. F. Tropical macrocytic anemia. *Lancet*, 235 : 416, 1938.

Microcytic, Hypochromic, and Nutritional Anemias

HEATH, C. W. *Anemia Due to Iron Deficiency*. Symposium on the Blood and Blood-Forming Organs. Madison, Univ. of Wisconsin Press, 1939.

HEATH, C. W., and PATEK, A. J., Jr. The anemia of iron deficiency. *Medicine*, 16 : 267, 1937.

MINOT, G. R. *Anemias of Nutritional Deficiency*. Symposium on the Blood and Blood-Forming Organs. Madison, Univ. of Wisconsin Press, 1939.

Aplastic Anemia

ISRAËLS, M. C. G., and WILKERSON, J. F. Etiology and prognosis of aplastic anemia. *Quart. J. Med.*, 9 : 163, 1940.

Anoxia; Oxygen Poisoning

ARMSTRONG, H. G. *Principles and Practice of Aviation Medicine*. Ed. 2. Baltimore, Williams and Wilkins, 1943.

BEAN, J. W. Effects of oxygen at increased pressure. *Physiol. Rev.*, 25 : 1, 1945.

BRAZIER, M. A. B. Effects of carbon dioxide on the nervous system in relation to anoxia. *Medicine*, 22 : 205, 1943.

GELLHORN, E., and LAMBERT, E. H. *The Vasomotor System in Anoxia and Asphyxia*. Chicago, Univ. of Illinois Press, 1940.

STADIE, W. C., et al. Oxygen poisoning. *Am. J. M. Sc.*, 207 : 84, 1944.

VAN LIERE, E. J. *Anoxia*. Chicago, Univ. of Chicago Press, 1942.

Methemoglobinemia; Sulfhemoglobinemia; Carbon Monoxide Poisoning; Agranulocytosis

BENSLEY, E. H., *et al.* Familial idiopathic methemoglobinemia. *Quart. J. Med.*, 7 : 325, 1938.

DAMESHEK, W. Leucopenia and Agranulocytosis. New York, Oxford Univ. Press, 1944.

DRINKER, C. K. Carbon Monoxide Asphyxia. London, Oxford Univ. Press, 1938.

FITZ-HUGH, T., Jr. Sensitivity reactions of the blood and bone marrow to certain drugs. *J. A. M. A.*, 111 : 1643, 1938.

HARROP, G. A., and WATERFIELD, R. L. Sulfhemoglobinemia. *J. A. M. A.*, 95 : 647, 1930.

KILLICK, E. M. Carbon monoxide anoxemia. *Physiol. Rev.*, 20 : 313, 1940.

Jaundice (Icterus)

(See references to Biliary Pathology, page 174.)

BARRON, E. S. G. Bilirubinemia. *Medicine*, 10 : 77, 1931.

OTTENBERG, R., and SPIEGEL, R. Nonobstructive jaundice due to infections and chemical agents. *Medicine*, 22 : 27, 1943.

STEIGMANN, F., and DYNIEWICZ, J. M. Urobilinogen determinations in the differential diagnosis of jaundice. *Gastroenterology*, 1 : 855, 1943.

WATSON, C. J. The bile pigments. *New England J. Med.*, 227 : 705, 1942.

Porphyrin; Photosensitivity

American Association for the Advancement of Science. Symposium on Blood, Heart and Circulation. Washington, 1940.

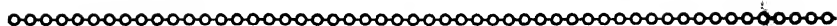
BLUM, H. F. Photodynamic Action and Diseases Caused by Light. New York, Reinhold, 1940.

DOBBINER, K., and RHOADS, C. P. The porphyrins in health and disease. *Physiol. Rev.*, 20 : 416, 1940.

WATSON, C. J. Porphyrins and Diseases of the Blood. Symposium on the Blood and Blood-Forming Organs. Madison, Univ. of Wisconsin Press, 1939.

CHAPTER VIII

INORGANIC SUBSTANCES



GENERAL FACTORS IN MINERAL METABOLISM

"It is significant that the more developed a science is, the less use it makes of history."
— MORRIS R. COHEN

ESSENTIAL NATURE AND REQUIREMENTS

All biological functions require suitable concentrations of water and inorganic electrolytes. The minerals, or inorganic substances, are present in tissues as ordinary salts, and as coordination complexes. The metallic trace elements of living material are largely in the form of complexes of carbohydrates, organic acids, and proteins, in which the metallic atom is part of a complex anion. The major fraction of the inorganic material of soft tissues and body fluids is in solution; but in bone it is deposited as a solid phase.

For successful growth, and the maintenance of health, the diet must contain a suitable assortment of minerals in a total concentration of approximately 4 per cent of the dry weight of the food. Growth is inhibited when the mineral content of the diet falls below this level, or when it is raised to 16 per cent of the dry weight; at a 32 per cent level death results. Continued drinking of water which contains 1 per cent or more calcium, magnesium, or sodium salt inhibits growth and causes irritation of the intestine and bladder. Prolonged mineral deficiency, in rats, incites increased catabolism of fat, decreased catabolism of carbohydrate, and a fall in the respiratory quotient. The essential minerals for mammals include calcium, cobalt, copper, iron, magnesium, manganese, potassium, sodium, and zinc, and chloride, iodide, and phosphate anions. The mineral elements differ from carbon, hydrogen, nitrogen, oxygen, and sulfur in that they need not be ingested in the form of organic compounds. The average daily intake of minerals for the human adult is approximately as follows, in meq.: sodium, 215; potassium, 75, calcium, 60; magnesium, 35; chloride, 215; phosphate, 105; and potential sulfate (chiefly in the form of amino acid sulfur), 90. Most of the sodium and chloride is ingested as table salt. Milk furnishes about 40 per cent of the food minerals; meat and eggs, about 25 per cent; cereals, 15 per cent; vegetables, 15 per cent; and fruit and nuts, 5 per cent.

ABSORPTION

Inorganic foods are absorbed chiefly by the small intestine, although water and the very diffusible monovalent ions can also permeate the stomach and large intestine. Prior to their absorption, salt solutions are adjusted to near isotonic concentration by admixture with gastro-intestinal secretions. The rate of absorption of inorganic ions is roughly in the order of the Hofmeister series (Table 11, page 50), the polyvalent ions being more slowly absorbed than the univalent ions. Magnesium sulfate, which has two divalent ions, is poorly absorbed and acts as a cathartic.

STORAGE

Absorbed minerals are transported to the tissues by the blood and lymph. The skeleton is the main depot for calcium, magnesium, sodium, carbonate, phosphate, and the trace minerals (aluminum, beryllium, gold, lead, radium, silver, strontium, tin, zinc, fluoride, titanate, and tungstate). Muscle is an important storehouse for magnesium, potassium, and sodium. The liver is especially concerned in the storage of bismuth, cadmium, chromium, cobalt, copper, gold, iron, manganese, nickel, silver, thallium, antimonate, arsenate, molybdate, selenate, and vanadate. The skin stores tin and arsenate, and the thyroid selectively removes iodide from the circulation. The heavy metals tend to be retained within the body for long periods; they are excreted very gradually.

The water content of the developing fetus falls rapidly, while its mineral concentration increases. Similar changes continue, at slower rates, throughout postnatal life. The total ash of the newborn infant is approximately 100 gm., as compared with 2,800 gm. for the average adult (65 kg. body weight). The ash content of the skin declines until puberty, and then increases slowly. Ash constitutes about 4.35 per cent of the body weight of the adult (5.2 per cent on a fat-free basis); it represents only 1 per cent of the average soft tissue, but 22 per cent of the skeleton (or from 50 to 60 per cent of dried defatted bone). The skeleton contains more than 80 per cent of the body ash, and 90 per cent of the inorganic cations. Another 10 per cent of the body ash, and 5 per cent of the cations are found in the muscles. The average weights of human organs (Table 95) are useful for computations in mineral metabolism.

MINERAL EQUILIBRIUM

The inorganic foods function chiefly as catalysts in biological processes. The only enzymes concerned in mineral metabolism are those which promote synthesis and catabolism of such compounds as dibromotyrosine, diiodotyrosine, thyroxine, chlorophyll, cupreins, and iron-porphyrin complexes, and the phosphorylases, phosphatases, and sulfatases (which

TABLE 95
APPROXIMATE WEIGHTS OF HUMAN ORGANS

	AT BIRTH		ADULT ¹	
	Grams	Per Cent of Body Weight	Grams	Per Cent of Body Weight
Muscle	750	23.5	26,700	41.0 ²
Adipose tissue	380	12.0	11,700	18.0 ²
Skeleton	500	15.5	9,700	15.0
Skin	370	11.5	5,000	7.7
Blood	245	7.6	4,950	7.6
Liver	140	4.5	1,600	2.5
Brain	400	12.5	1,350	2.1
Intestine	50	1.5	1,200	1.8
Lungs	55	1.7	850	1.3
Mammary glands	3	0.1	500	0.77
Heart	20	0.6	300	0.45
Kidneys	25	0.8	300	0.45
Peripheral nervous system			195	0.3
Stomach	25	0.8	150	0.23
Spleen	10	0.3	150	0.23
Pancreas	3	0.1	105	0.16
Esophagus			70	0.11
Bladder			50	0.08
Uterus	4	0.12	50	0.08
Salivary glands	3	0.1	45	0.07
Testes	2	0.06	40	0.06
Spinal cord	3.5	0.1	30	0.05
Thyroid gland	2	0.06	25	0.04
Eyes	5	0.15	25	0.04
Ovaries	0.3	0.01	20	0.03
Thymus	10	0.3	20	0.03
Prostate	0.5	0.015	15	0.02
Adrenal glands	7	0.2	12	0.02
Pituitary gland	0.3	0.01	0.6	0.001
Pineal gland	0.01	0.0003	0.15	0.0002
Parathyroid glands			0.12	0.0002
Total body weight	3,200		65,000 ⁴	

¹ With the exception of the reproductive organs, the averages include both sexes.

² Male, 43 per cent; female, 39 per cent.

³ Male, 15 per cent; female, 21 per cent.

⁴ This represents the average male weight; the average weight of the female is about 57 kg.

form phosphate and sulfate esters of organic hydroxy compounds, or hydrolyze these esters).

The normal adult is in a state of mineral equilibrium, in which the intake and excretion of individual inorganic substances are balanced.

Individual body fluids are also maintained in a state of osmotic and acid-base equilibrium. The very high osmotic pressure in certain marine animals depends on organic crystalloids. In normal human blood plasma, the determined total base, that is, the sum of inorganic cations, is 154 meq. per liter, and the total anion is 154 meq. per liter (page 33). Throughout life, the plasma total base varies only between 120 and 180 meq. The normal electrolyte concentration is maintained by variation in mineral excretion, and, in conditions of electrolyte imbalance, by retention or excretion of the water of extracellular fluids. The concentration of water in the body is more important to life than the maintenance of a constant fluid volume. Mineral equilibria are, therefore, measured by balance experiments rather than by determinations of mineral concentrations in tissues or body fluids.

Growing infants and children exhibit positive mineral balances. Rapid retention occurs during the last two months of fetal life, at which time more than 1 gm. ash (representing 23 and 15 meq. of cations and anions, respectively) are deposited daily. The mineral deficiencies of premature infants predispose to anemia and rachitis. Calcium and phosphate, which are retained by the growing infant in relatively large quantities, are most efficiently provided by milk. Hence, the growth of infants is proportional to the quantity of milk they consume. Modified cow's milk has a greater mineral content than does breast milk, but the minerals of the latter are absorbed more efficiently.

Excretory organs are of prime importance in mineral equilibria; they selectively excrete cations and anions. The kidney conserves certain cations by manufacturing ammonium, and it can spare mineral anions by excreting bicarbonate. Diarrhea increases the intestinal excretion of potassium, sodium, and chloride; dehydration is accompanied by an increased urinary excretion of cations; and alkalosis shifts calcium and phosphate excretion from the kidney to the intestine. Storage depots participate in mineral equilibria by replenishing deficiencies in the tissue fluids. The bones contribute calcium, magnesium, carbonate and phosphate; the extracellular fluids, sodium, bicarbonate, and chloride; and the intracellular fluids, potassium, phosphate, and sulfate.

EXCRETION

The intestine is the major excretory organ for aluminum, beryllium, calcium, copper, iron, magnesium, manganese, silver, strontium, tin, zinc, fluoride, molybdate, and tungstate; it shares with the kidney the excretion of lead, nickel, arsenate, phosphate, silicate, and vanadate. The small intestine, colon, and liver are concerned in mineral excretion. Disease usually causes much less impairment of intestinal mineral excretion than of mineral absorption.

The kidneys are the chief excretory organs for bismuth, chromium,

cobalt, gold, lithium, mercury, potassium, sodium, thallium, uranyl, antimonate, borate, bromide, chloride, fluoride, iodide, selenate, sulfate, and titanate. The daily urine of an adult human contains about 55 gm. of solids, 22.5 gm. of which are inorganic salts.

Only small quantities of minerals are excreted normally in sweat; but lactation causes very appreciable mineral losses.

URINE SECRETION

In contrast to the gastro-intestinal glands, whose secretions remain in osmotic equilibrium with the blood plasma, the renal tubules perform osmotic work by concentrating the crystalloids of the glomerular blood plasma ultrafiltrate. The average renal concentration of various nitrogenous substances may be found in Table 77, page 434, and the daily urinary output and concentration of inorganic substances are summarized in Table 96. Concentration tests of renal function have been discussed on page 450.

TABLE 96

INORGANIC SUBSTANCES OF NORMAL URINE

	GRAMS IN AVERAGE DAILY URINE	APPROXIMATE CONCENTRATION BY THE KIDNEY
Calcium	0.2	2 times
Chloride	7.0	2 times
Citrate	0.3	
Copper	0.00025	
Iodine	0.00005	
Iron	0.0002	
Magnesium	0.2	6 times
Phosphorus (phosphate) .	1.0	25 times
Potassium	3.0	12 times
Sodium	5.0	1 time
Sulfur (inorganic sulfate) .	0.8	50 times
Volume	1,200 ml.	

A human kidney contains slightly more than one million nephrons, with a total glomerular capillary surface of more than 1 square meter. All nephrons in man and the dog apparently function continually. The plasma is first ultrafiltered from its colloidal constituents in the glomeruli, and this filtrate is subsequently concentrated in the tubules by active reabsorption of water, calcium, magnesium, potassium, sodium, bicarbonate, chloride, glucose, ascorbic acid, and amino acids. There is, in addition, a smaller "passive" tubular reabsorption of galactose, phos-

phate, sulfate, urea, and uric acid. Water and glucose are reabsorbed chiefly in the proximal tubules, and the inorganic ions in the distal tubules.

The average renal blood flow in man is approximately 1,300 ml. per minute, or about 32 per cent of the basal cardiac output. Diodrast clearance (by tubular excretion and glomerular filtration) measures the renal plasma flow, which is approximately 700 ml. per minute. The circulation through the kidney is rather constant in the face of changing arterial pressure, and it is not dependent on innervation. According to inulin clearance, the human glomeruli normally filter about 18 per cent of the plasma flow through the kidney; in man, the glomerular filtrate amounts daily to approximately 180 liters (or sixty-eight times the plasma volume). When the renal blood flow is decreased by adrenaline, or increased by anterior pituitary extract, pyrogen, or thyroglobulin, the glomerular filtration rate is altered, but the number of functioning glomeruli is unchanged. During tubular concentration, approximately 179 liters of water, 1,100 gm. sodium chloride, 420 gm. sodium bicarbonate, and 140 gm. glucose are reabsorbed daily. The maximal rate of tubular reabsorption (T_m) of glucose provides an estimate of the reabsorptive capacity of the tubules; in the normal adult it is about 345 mg. per minute. In addition to the primary renal mechanisms, the proximal convoluted tubules secrete ammonium cations, and they excrete such foreign substances as phenol red, *p*-amino and *p*-hydroxy derivatives of hippuric acid, and some organic iodine compounds (diodrast). The maximal tubular excretion of diodrast (52 mg. of iodine per minute in normal men) is a measure of the number of active tubules. Hypophysectomy lowers the diodrast T_m , and the administration of testosterone propionate increases it. Thyroxine raises both diodrast T_m and glucose T_m . (See pages 452 to 454 for a discussion of clearance tests.)

During urinary filtration and reabsorption, the oncotic pressure of the plasma proteins (normally, 28 ± 2 mm. mercury) operates in opposition to the hydrostatic blood pressure (about 32 mm. mercury in general capillaries and 75 mm. in glomerular arterioles). The urinary output is increased by elevation of the renal blood pressure, increase of the blood flow through the kidneys, or by increase in the permeability of the glomerular capillaries. Nervous and hormonal secretory influences operate partly by altering these factors. Section of the renal innervation results in the secretion of a large volume of dilute urine (polyuria or diuresis). Replacement of plasma by physiologic sodium chloride solution results in increased urine output. Elevated oncotic pressure of the plasma, and increased hydrostatic pressure within the tubular lumen, accelerate reabsorption and reduce the urinary output (oliguria).

The chemical composition of the blood plasma and the nature of the diet influence the urine volume. Normal fluctuations in urinary output are largely attributable to varying water intake. Water deficit hinders the excretion of crystalloids, because of the definitely limited concentrating

ability of the kidney. Excessive excretion of cations, as in metabolic acidosis, results in diuresis. The primary renal function is the maintenance of the normal composition of blood plasma, and especially of the total plasma base. Hence, urine secretion is affected more readily by changes in plasma composition than by the volume of the blood or extracellular fluid.

METABOLISM OF WATER

*"A little learning is a dangerous thing;
Drink deep, or taste not the Pierian spring;
There shallow draughts intoxicate the brain,
And drinking largely sobers us again."*

— ALEXANDER POPE

The body water is present in the free state and in association with ions, crystalloids, and colloids. The so-called "bound water," adsorbed on colloids, constitutes less than 4 per cent of the total body water. Tissue water can be determined by weight loss on suitable drying. Water is distributed in the body as intracellular and extracellular fluid; the latter is contained partly within the fixed tissues, and partly in circulating fluids and secretions. Body water has numerous functions, such as ionization, solvation, circulation, and temperature regulation. Some of its chemical activities are due to the ease with which it forms aquo complexes.

WATER INTAKE

The general sources of body water are ingested beverages, the water of solid foods, and the water of metabolism which is formed by the combustion of foods. The average daily water requirement of adults is about 4 per cent of the body weight. Drinking water ordinarily provides from 1,000 to 1,500 ml. daily; most of the remainder is provided by other beverages and by solid foods. Fruits and vegetables contain large quantities of water. For example, asparagus, cabbage, cauliflower, celery, cucumbers, lettuce, melons, onions, peppers, squash, string beans, and tomatoes contain from 90 to 97 per cent water; meat has approximately 70 per cent. The water of metabolism satisfies only a small fraction of the total requirement of human beings, but it is of prime importance in hibernating and desert-inhabiting animals. About 12 ml. water are formed for each 100 calories of energy output. Oxidation of 100 gm. portions of fat, carbohydrate, and protein yields 107, 55, and 41 ml. water, respectively. The water requirement is increased by exercise, ingestion of food, and by high temperatures or humidity of the air. The requirement is related to the body surface and metabolic rate (approximately 1 ml. per calorie); it is raised by fever and by increased intake of protein or sodium chloride. Control of water intake is related to thirst.

ABSORPTION

Water is absorbed to some extent in the stomach, but much more rapidly through the small intestine into the blood and lymph. Considerable quantities can also be absorbed from fluid enemas introduced slowly into the large intestine; water is administered in this manner to patients afflicted with vomiting or with intestinal obstruction. Water can be supplied parenterally by the subcutaneous injection of isotonic saline or glucose solutions. Injected solutions are adjusted to near isotonic concentrations at the site of injection before they are absorbed.

TRANSPORTATION; BLOOD VOLUME

About 10 per cent of the body water (or approximately one third of the extracellular water of the body) is normally present in the blood plasma and other circulating fluids. The large volume of isotonic fluid absorbed postprandially from the intestine is transported rapidly to the tissue fluids. This extensive transfer is not revealed by studies of the plasma water; the plasma composition is maintained in normal animals by rapid interchange with other extracellular fluids. The blood volume of the adult human is equivalent to approximately 7.6 per cent of the body weight, although it is more nearly proportional to the body surface. The erythrocyte volume is normally about 35 ml. per kg. of body weight; it can be determined experimentally from the blood carbon monoxide concentration after inhalation of a definite quantity of the gas, or by labeling the erythrocytes with radioactive iron. The plasma volume, which is normally about 41 ml. per kg. of body weight, can be estimated from the concentration of intravenously injected hemoglobin or vital dyes (Evans blue; vital red). Estimates made in this fashion are approximately 10 per cent higher than those based on erythrocyte volume, because the dyes tend to enter the hepatic lymph as adsorption compounds of the plasma proteins. (See embolic effect, page 56.)

About thirty minutes after the ingestion of water, the blood volume temporarily increases as much as 15 per cent while water is being transported to the tissues; somewhat later, a second slight dilution results from transport of the water to excretory organs. Similar effects follow the intravenous injection of an isotonic solution. The plasma protein concentration is intimately related to transudation of fluid and alteration of blood volume. The liver is an emergency regulator of blood volume and pressure; it temporarily stores water entering from the intestine.

DISTRIBUTION OF BODY WATER

Water constitutes about 63 per cent of the body weight of the adult human; the water content of various human tissues is presented in Table 97. Cerebrospinal fluid and sweat have the greatest concentrations

TABLE 97
APPROXIMATE WATER DISTRIBUTION
IN THE HUMAN ADULT¹

	PER CENT WATER IN TISSUE	KG. WATER IN TISSUE	PER CENT OF TOTAL BODY WATER
Muscle	75	20.02	48.6
Skeleton	46 ^{2, 3}	4.46	10.8
Blood	82 ⁴	4.06	9.9
Adipose tissue .	30 ³	3.51	8.5
Skin	70 ³	3.50	8.5
Liver	74 ³	1.18	2.8
Brain	76 ⁵	1.03	2.5
Intestine	75	0.90	2.2
Lungs	78	0.66	1.6
Heart	79	0.24	0.58
Kidneys	81	0.24	0.58
Stomach	75	0.11	0.27
Spleen	77	0.12	0.29
Pancreas	73	0.08	0.19
Other tissues . .		1.07	2.6
Entire body . .	63	41.2 ⁶	100

¹ Based on values in column 4 of Table 95, page 570.

² Very variable.

³ Bone freed of marrow contains about 22.5 per cent of water.

⁴ Erythrocytes, 65 per cent; plasma, 92 per cent.

⁵ Gray matter, 84 per cent; white matter, 70 per cent; spinal cord, 75 per cent.

⁶ The total water of a 3.2 kg. newborn human is about 2.3 kg., corresponding to 72 per cent of water.

of water (about 99 per cent), while tooth enamel has the lowest (3 per cent). The water content of bone and adipose tissue is low, and fat deposits do not replace body water to any great extent. Tissues which have a variable water content include the liver, bone, skin, and adipose tissue. Muscles contain one half of the body water; blood, bone, fat, and skin each have from 8.5 to 10 per cent of the total water content.

The water content of the fetus at six weeks is 97.5 per cent; it decreases rapidly during development until the third week of infant life. A much slower dehydration continues throughout life. Rapidly growing neoplasms have greater water concentrations than do normal adult tissues.

EXTRACELLULAR WATER AND STORAGE

Most of the water in the fetus is extracellular; the intracellular fraction increases with age. In the adult, 71 per cent (29 liters) of the body water is intracellular. Another 19 per cent (8 liters) of the body water of the

adult consists of extracellular fluid in the tissues; and 10 per cent (4 liters) is circulating extracellular fluid (chiefly blood plasma). About 16 per cent of the muscle water and 21 per cent of the liver water are extracellular, as estimated from the electrolyte distribution. The remainder of the muscle water is present largely in the fibers and sarcolemma. Extracellular water can be estimated by determining the plasma concentration of injected sodium salt (bromide, iodide, nitrate, sulfate, or thiocyanate), radioactive sodium salt, sucrose, or inulin. These substances, and bicarbonate, are distributed rapidly throughout extracellular body water.

Studies with deuterium oxide indicate that water equilibrium between the capillaries and extracellular space is established within thirty seconds; the intracellular water is in equilibrium in thirty minutes; and water molecules remain in the human body about three weeks. The extracellular fluid of tissues constitutes a reservoir for water and electrolytes. The 8 or 9 liters of daily digestive secretions (Table 27, page 132) are roughly equal to the entire volume of the extracellular fluid from which the water is mobilized. According to deuterium oxide studies in the guinea pig, the hourly placental transfer of water equals twice the weight of the fetus. While the volume of extracellular fluid fluctuates, its composition remains rather constant. Injection of isotonic saline solution increases the extracellular fluid volume; alkaline salt solutions are more effective than acid salt solutions. Intravenously injected isotonic fluids are transferred rapidly to the extracellular tissue fluid, and are excreted after they have again traversed the blood stream. Parenteral introduction of hypertonic saline or glucose solutions necessitates osmotic exchange and water transfer between the intracellular and the extracellular fractions of fluid in order to equalize their osmotic pressures. The cerebrospinal fluid pressure is lowered for some hours following the intravenous injection of 50 per cent sucrose solution.

The volume of extracellular fluid increases whenever suitable electrolytes are retained. Connective tissue is the chief receptor for extracellular fluid. The liver temporarily stores fluid absorbed from the intestinal tract. This organ is only an emergency regulator; it stores water for shorter periods than do muscles, subcutaneous tissues, or skin. Thyroid tends to prevent accumulation of extracellular water and protein.

WATER BALANCE

The water balance is correlated with the metabolism of organic and inorganic foods. High carbohydrate diets stimulate slight water retention; for each gram of glycogen and protein deposited in tissues, approximately 1.5 and 3 ml. of water are retained, respectively. The deposition of fat does not lead to appreciable water retention. A sufficiently high fat intake incites ketosis and a marked water loss which accompanies the excretion of extra base. Loss of 10 per cent of the body water causes serious symp-

toms of dehydration, and a 20 per cent loss can prove fatal. These effects may be contrasted with the recovery of starved animals after losing 40 per cent of their body weight, most of their fat and glycogen, and one half their protein.

Loss of body water is usually accompanied by loss of base; the excretion of sodium and chloride closely parallels that of water. When tissue protein catabolism becomes excessive, losses of potassium, sodium, chloride, and both intracellular and extracellular water result. Increased ingestion of salts, without a correspondingly large water intake, causes dehydration. Acidifying measures stimulate the excretion of base and water, while the administration of alkali has an opposite effect. The ingestion of sodium chloride and a liberal quantity of water leads to increased extracellular fluid volume, especially in children, starving adults, and nephritic patients. Excessive intake of calcium or potassium stimulates water loss. A sudden lowering of the sodium intake causes rapid loss of body water.

EXCRETION

The principal water-excreting organs are the kidneys, lungs, skin, intestine, and active mammary gland (Table 98). The feces of normal adults contain approximately 75 per cent water, and those of infants from

TABLE 98
EXCRETION OF WATER

	HUMAN ADULT	
	Average Daily Output (Milliliters)	Total Water Loss (Per Cent)
Urine	1,200 \pm 350	50
Skin		
Insensible perspiration .	500 \pm 200	20
Sweat	300 \pm 200	12.5
Lungs	300 \pm 50	12.5
Feces	100 \pm 50	5
Total	2,400 \pm 850	
	Approximate Daily Urine Volumes (Milliliters)	
Newborn	50 \pm 40	
Infant	450 \pm 150	
Children (to 10 years) . .	750 \pm 250	
Adult	1,200 \pm 350	

80 to 85 per cent. Fecal excretion normally accounts for only 5 per cent of the total water loss, but it is markedly increased by diarrhea or catharsis, and less drastically by the ingestion of such indigestible hydrophilic colloids as agar and hemicellulose.

The air exhaled from the lungs is almost saturated with water vapor. Under basal metabolic conditions, about one eighth of the daily water is excreted by the lungs; the quantity depends on the temperature and humidity of the air, and on the respiratory rate. Approximately one fourth of the heat production of humans is dissipated by vaporization of water at the lung and skin surfaces. Increased environmental temperature raises this fraction, while increased humidity has an opposite effect. The total insensible perspiration from the lungs and skin is proportional to the basal metabolic rate. It is decreased by dehydration. About one fifth of the daily water excretion occurs as insensible perspiration of the skin, and another one eighth as sweat. These figures apply only to basal metabolic conditions; under other circumstances, the volume of sweat is variable. At sufficiently high environmental temperatures and humidities, it can exceed 10 liters per day. Sweat is secreted whenever additional cooling is required to preserve the body temperature, as in fever, and also as the result of nervous excitation. Atropine paralyzes the cholinergic nerve endings of the sweat glands. Hypothyroid conditions cause a dry skin, while hyperthyroidism stimulates perspiration (through calorogenic action). When sweating is inadequate, the body temperature rises and heat stroke can occur.

Ordinarily, the secretion of urine accounts for one half of the total water output; the urinary volume is proportional to the fluid intake, and to the quantities of electrolytes which are excreted in the urine. It is inversely proportional to the quantity of water excreted by the skin and the gastrointestinal tract. The urine volume is increased by nervousness or excitement, and it is decreased by muscular exercise. The nocturnal output is usually one third of the day volume. *Diuretics* increase the urinary volume. Ingested water is largely eliminated in the urine within two hours, and normally it acts as a diuretic. Subcutaneously injected saline solution is absorbed slowly, and exerts only a slight diuretic action, whereas intravenously injected hypertonic saline or sugar solutions are powerful diuretics. The ingestion of protein, urea, or of sufficient inorganic salts to exceed the concentrating ability of the kidney, causes an increased urine output. The urine volume, therefore, increases for an hour or two after an average meal. Anions, other than chloride, form a Hofmeister diuretic series; nitrates are effective diuretics. The purine diuretics (theophylline, theobromine, caffeine) act on the renal blood vessels. The mercurial diuretics diminish tubular reabsorption. Digitalis acts extrarenally, by improving circulatory efficiency in cardiac patients. Acidifying diuretics (page 40), and conditions of metabolic acidosis, cause diuresis by increasing the excretion of base; the ingestion of alkali tends to diminish water

secretion. Thyroglobulin, androgens, and parathormone have diuretic effects, while castration leads to water retention. Insulin exerts an indirect antidiuretic action by inhibiting ketosis and the accompanying loss of electrolytes. Desoxycorticosterone decreases the excretion of sodium chloride and inhibits water loss in adrenal cortical insufficiency. Adrenaline causes vasoconstriction, and a temporary decrease in urine volume. The most powerful antidiuretic hormone is pitressin, of the posterior pituitary. It can act directly on the distal tubules of denervated isolated kidneys, and it is necessary for 20 per cent of the normal reabsorption of water in these structures.

METABOLISM OF THE PREDOMINANT MINERALS

"Brighter stars will rise on some voyager of the future — some great Ulysses of the realms of thought — than shine on us." — SIR JAMES G. FRAZER

MONOVALENT IONS

Sodium

This cation constitutes the largest fraction of the total base of body fluids; it is, therefore, important in osmotic equilibria. In living material, sodium is present almost entirely as the cation, in association with water and with chloride and bicarbonate anions. It can be determined in tissues and body fluids, after wet ashing or deproteinization with trichloroacetic acid, by precipitation as uranyl zinc sodium acetate or as sodium pyroantimonate in alcoholic solution; the former can be titrated with sodium hydroxide; the latter, with thiosulfate.

The dietary sodium of human beings is provided by table salt, and a variety of foods (Table 99). The adult ingests from 4 to 5 gm. sodium daily in the form of from 10 to 12 gm. table salt; this is equal to 10 per cent of the total sodium in the extracellular fluid of the body. Table salt renders foods palatable and aids the secretion of digestive juices.

Sodium cations are absorbed to some extent by the pyloric mucosa, and rapidly by the normal small intestine, but somewhat less readily in adrenalectomized animals. The mineral is transported by the blood and lymph, and is distributed rapidly throughout the extracellular tissue fluids without significant elevation of the normal plasma level (335 ± 10 mg. per cent). Human erythrocytes contain only 23 mg. per cent sodium; the erythrocytes of rats and rabbits have low sodium concentrations while those of the dog, cat, sheep, and cow have much larger concentrations. Administered Na^{24} enters the erythrocytes of the dog slowly, but does not penetrate rat, rabbit, or human erythrocytes as readily. Serum sodium is lowered slightly by starvation, and more drastically by adrenal cortical insufficiency and heat cramps.

About 80 per cent of sodium storage is in the extracellular fluid; the

TABLE 99
COMMON FOODS WITH HIGH CONTENT OF
PREDOMINANT MINERALS

(Approximate Concentration in Mg. Per Cent)

SODIUM		POTASSIUM		CHLORIDE	
Butter	800	Olives	1,500	Butter	1,200
Cheese, clams	600	Molasses	1,350	Clams, rye bread	1,030
Oysters	450	Raisins	820	Cheese	880
Bread	400	Peanuts	650	Oysters	600
Egg white	170	Parsnips, spinach	530	Graham crackers	530
Eggs	140	Wheat	470	Bread	350
Celery, olives, raisins	130	Potatoes, rye	450	Molasses	320
Beets	100	Broccoli, oatmeal, sweet potato	400	Fish	180
Beef, spinach	85	Banana, mushrooms	390	Celery, egg white	160
Carrots	75	Beets, turnips	350	Bananas, coconut	120
Cauliflower, fish, oatmeal	70	Beef, lettuce, walnuts	330	Eggs, milk	110
Pumpkin, turnips	65	Pumpkin	320	Egg yolk, sweet potato	95
Broccoli, melons	60	Carrots, fish	310	Cream, raisins	80
Egg yolk	55	Cocoanut, figs	300	Beef, lettuce, spinach	75
Milk, peanuts	50			Oatmeal, wheat	70
CALCIUM		MAGNESIUM		TOTAL PHOSPHATE (AS P)	
Cream cheese	930	Chocolate	290	Cheese	700
Molasses	260	Peanuts	180	Egg yolk	600
Ice cream	150	Pecans	150	Bran flakes, yeast	550
Egg yolk	130	Shredded wheat	140	Chocolate	450
Broccoli, cow's milk, cau- liflower	120	Walnuts, wheat	130	Oatmeal, puffed wheat	420
Maple syrup	110	Oatmeal	120	Peanuts	400
Cream	100	Clams, raisins	85	Wheat	370
Chocolate, pecans, wal- nuts	90	Molasses	70	Walnuts	360
Cottage cheese, spinach	80	Spinach	50	Pecans	330
Bran flakes, celery, eggs, peanuts	70	(Dairy products, fruits, and vegetables have low concentrations.)		Shredded wheat	320
Oatmeal, raisins, turnips	65			Ham	270
Beans (cooked), oysters, wheat	50			Cottage cheese	260
Breast milk	30			Whole wheat bread	250
				Fish, turkey	230
				Eggs, liver, pork	220
				Chicken, lamb, lobster	210
				Beef	200

most important sodium depots are the skin, subcutaneous tissue, muscle, and bony skeleton. The largest sodium concentrations are in cartilage, blood plasma, and lymph. The smallest concentrations are found in gastric juice, muscle, pancreas, milk, and saliva (Table 100). Skeletal muscle has only one half as much sodium as smooth or cardiac muscle; muscles gain sodium during activity, and lose it during recovery. In nutritional muscular dystrophy the sodium concentration of the affected muscles is increased.

Transfer of sodium accompanies that of chloride; it is slower than water transfer, since injected radioactive sodium ions require about 2 hours to become equilibrated with extracellular fluid in normal persons, and even longer with edema fluid. Ingestion of 40 gm. sodium chloride daily causes sufficient increase in the tissue fluid of the normal adult to produce slight

TABLE 100

A. APPROXIMATE TISSUE CONCENTRATIONS OF
PREDOMINANT MINERALS

(Mg. Per Cent)

TISSUE	SODIUM	POTASSIUM	CHLORIDE	CALCIUM	MAGNE- SIUM	INORGANIC PHOSPHATE (AS P)	INORGANIC SULFATE (AS S)
Blood	175 ± 10	210	280 ± 15	5	4.3	3 ± 1	1 ± 0.3
Serum (plasma)	335 ± 10	20 ± 2	365 ± 15	10.3 ± 0.3 ¹	2.4 ± 0.7	3.7 ± 0.8 ²	1.3 ± 0.5
Erythrocytes	23	420	190	0	6.6 ± 1.2	0	
Aqueous humor	300	13	440	5	2.5	2	
Bile (hepatic)	320	19	350	10	0.5	14	
Cerebrospinal fluid	325 ± 20	20 ± 2	440 ± 15	5 ± 0.5	3 ± 0.5	1.6 ± 0.4	
Lymph	290	14	420	10	3	3.6	
Milk, human	13	50	35	32	5	15	
Milk, cow	60	150	115	125	14	85	
Saliva	20	100	42	6	2	18	
Brain	150	290	130	10.5	14	7	10
Cartilage	550	235	250	40	11	10	
Heart	170	230	125	9	16		
Intestine		270	60	13	7.5		
Kidney	165	165	210	19	20		7
Liver	150	170	125	10	17		20
Lung	245	150	255	16	7.5		10
Muscle (striated)	70	280	60	7	21	18	6
Pancreas	80	200	160	15	17		
Skeleton	160	55	170	9,900 V ³	95	4,550 V	
Skin	200	60	260	11	6		
Spleen			155	10	15		
Teeth							
Dentin				24,500	800	12,000	
Enamel		300		34,500	250	16,500	
Testis			230	9	9.5	10	
Thyroid			170	34	9.5		
Total grams in en- tire body						Total P	Total S
Newborn	4.7	5.1	5	23.6	0.7	13.8	6.3
Adult (65 kg.)	60	140	80	1,075	20	620	105

B. DISTRIBUTION OF PHOSPHORUS IN BLOOD

(Mg. Per Cent)

	TOTAL P	LIPIDE P	ACID SOLUBLE P	
			Ester P	Inorganic P
Blood	38 ± 3	12 ± 1	23 ± 2	3
Serum	13	8	1	3.7 ± 0.8
Erythrocytes	68	17	50 ⁴	0

¹ Infant, 11 mg. per cent.² Infant, 5 ± 1.0 mg. per cent.³ Dried, defatted bones contain 20 to 25 per cent calcium, and correspondingly greater concentrations of other minerals.⁴ At birth, 80 mg. per cent; at two years, 90 mg. per cent. In erythrocytes of the adult, this fraction includes 30 mg. per cent as diphosphoglycerate, 17.5 mg. per cent as adenosine triphosphate, 0.5 mg. per cent as coenzyme II, and 0.2 mg. per cent as coenzyme I.

edema. When the water intake is limited and a diet high in sodium chloride is administered, the osmotic pressure of the body fluids increases and salt fever results, especially in children. The sodium concentration of the body and the volume of the extracellular fluid decrease with age.

About 5 gm. sodium are excreted daily in the urine of the human adult; this excretion accounts for 95 per cent or more of the total sodium output, the remainder being excreted in the sweat and feces. Diarrhea reverses the proportions excreted in the urine and feces; profuse sweating and fasting cause drastic reduction of the urinary sodium. Above a limiting plasma level, the kidney excretes sodium rapidly; but the kidney is unable to concentrate it appreciably. Desoxycorticosterone is necessary for the reabsorption of sodium by the proximal renal tubules; this hormone prevents excessive urinary loss of sodium and causes its retention. Progesterone, corticosterone, and dehydrocorticosterone exert similar activity, while pitressin and cortical hormones with hydroxyl radical at carbon 17 have an opposite effect. Administration of desoxycorticosterone and sodium (as chloride, bicarbonate, or citrate) assists restoration of sodium to the extracellular fluids, and causes clinical improvement in Addison's disease. Estrogens tend to produce sodium retention, but they are toxic to adrenalectomized animals. A prolonged high sodium intake causes renal hypertrophy in animals.

Potassium

This cation is a typical constituent of intracellular fluid, in which it is closely associated with phosphate, proteins, bicarbonate, and chloride. Potassium can be determined in tissues and biological fluids, after wet ashing or deproteinization, by precipitation as potassium cobaltinitrite, and titration of the latter with permanganate, or colorimetric determination of the cobalt by means of the green compound which it forms with choline and potassium ferrocyanide.

The average daily intake of potassium by the human adult is 3 gm.; this quantity is twice the requirement for maintenance and growth. About one third of the dietary potassium is derived from milk, another third from vegetables, and some of the remainder from meat (Table 99). Experimental potassium deficiency in rats causes slow growth, thin hair, necrosis of cardiac muscle, renal hypertrophy with tubular damage, ascites, coma, and death.

Potassium is absorbed rapidly from the intestine, and it is transported by the blood and lymph. Human serum contains 20 ± 2 mg. per cent, and the erythrocytes 420 mg. per cent. The potassium content of erythrocytes is higher in young animals than in adults. In preserved samples of blood, the erythrocytic potassium gradually diffuses into the plasma. Adrenal cortical insufficiency raises the serum potassium to approximately 60 mg. per cent. From 20 to 25 gm. potassium chloride must be ingested

by a normal man to cause appreciable increase in the serum potassium. Toxic doses of potassium salts raise the plasma level, depress neuromuscular irritability, and cause death. In severe adrenal or renal insufficiency, administration of potassium salt can increase the serum potassium to 30 mg. per cent with production of toxic symptoms. Large doses of insulin or glucose lower the serum potassium level; asphyxia increases it markedly.

Potassium differs from sodium and chloride in regard to its rapid entrance into cells, which is controlled by intracellular metabolic processes. Administration of radioactive potassium (K^{42}) to mammals shows only slow interchange with the brain, testis, and erythrocyte potassium, but the potassium of the liver, kidney, lung, gastro-intestinal tract, and muscle is exchanged rapidly. Fluoride and iodoacetate increase erythrocyte permeability to potassium through accumulation of intermediate metabolites within the cells. Stimulation of muscle facilitates the entrance of potassium into the muscle cells. Potassium is distributed uniformly in the muscle fiber. The myosin of muscles adsorbs potassium preferentially from a mixture of sodium and potassium phosphates. Experiments with K^{42} indicate that approximately two thirds of the muscle potassium is bound in non-diffusible form. There is less potassium in smooth or cardiac muscle than in striated muscle, brain, or intestine. The potassium concentration of bone, skin, and body fluids is low. Three fourths of the body potassium is in the muscles, and retention of this cation is associated with an increase in protein and body weight. The liver temporarily stores potassium absorbed from the intestine, as shown by K^{42} administration. The muscles assimilate the mineral more slowly. Potassium is apparently deposited in the liver cells during glycogenesis, and it is liberated from them during glycogenolysis. Intravenous injections of adrenaline rapidly mobilize liver potassium. During adrenal cortical insufficiency there is an increase in muscle potassium which is not shared by the brain or liver. Desoxycorticosterone mobilizes muscle potassium; this activity may be fundamental to the retention of sodium and chloride, which results when the hormone is injected into adrenalectomized animals. The cardiac glycosides exhibit somewhat similar effects. Changes in the mineral composition of the extracellular fluids do not easily alter the potassium content of cells; the intracellular potassium is partly diffusible and partly bound to colloids. Some potassium and calcium leave the muscle cell during muscular activity, acidosis, fasting, and increased endogenous protein catabolism. The potassium content of dystrophic or atrophic muscle is low. Administration of potassium salts benefits familial periodic paralysis and myasthenia gravis, while the muscles of patients with myotonia are hypersensitive to potassium.

About 90 per cent of the potassium excretion normally occurs in the urine; most of the remainder is excreted in the feces. The renal threshold for potassium is approximately 14 mg. per cent in the plasma. Potassium is

less intimately associated with water than is sodium, and the kidney normally concentrates potassium about twelve times; the concentrating ability is depressed in adrenal cortical insufficiency. The daily excretion of approximately 3 gm. potassium represents the excess intake and the potassium derived from tissue catabolism. Potassium excretion is raised by acidosis, administration of desoxycorticosterone or estrogens, and by increased endogenous catabolism; it is decreased by desoxycorticosterone deficit. Since potassium is not stored readily in the extracellular fluids, administered potassium salts tend to be excreted rapidly and to act as diuretics in normal animals.

Chloride

The distribution of chloride in animals roughly parallels that of sodium, and both ions are important in osmotic equilibria. Chloride is determined, after tungstic acid deproteinization or wet ashing, by precipitation with silver nitrate and titration of the excess silver with standard thiocyanate solution (ferric ammonium sulfate being used as indicator).

More dietary chloride is derived from dairy products and meat than from vegetables or fruits. The chloride content of foods is roughly proportional to the sodium content, and inversely proportional to the potassium content (Table 99, page 581). The principal source of dietary chloride is table salt. The average total chloride intake of the human adult is from 7 to 8 gm. daily. A daily intake below 3 gm. can retard growth, but clinical deficiencies are rare. The chloride anion acts as a coenzyme to digestive amylases. Chlorides are rapidly absorbed from the intestine and transported to the extracellular fluid by the blood and lymph. Absorption of chloride is decreased in adrenal cortical insufficiency, and is restored to normal by desoxycorticosterone.

Blood serum of humans normally contains 365 ± 15 mg. per cent, and the erythrocytes about 190 mg. per cent chloride. Radioactive chloride shows complete interchange across the human erythrocyte membrane within 10 minutes. Owing to the anion migration (page 23), and changes in erythrocyte volume, clinical chloride determinations are usually made on plasma, which has been separated from blood collected under oil. The plasma chloride level is affected only slightly by the ingestion of chlorides; it decreases about 7 mg. per cent at the height of gastric secretion. Plasma chloride diminishes during starvation, adrenal cortical insufficiency, and excessive vomiting.

The erythrocytes and a large proportion of the cells in the gastric mucosa, connective tissue, testes, and lungs have higher chloride concentrations than other mammalian cells (Table 100, page 582). The tendency of this anion to accompany extracellular fluid accounts for the high concentrations in the skin, skeleton, and lung. Parenterally injected chloride is transferred rapidly to the extracellular fluid, which normally contains

about 80 per cent of the body chloride. The low chloride levels in muscle and intestine correspond to the impermeability of these cells to chloride anions, and to their small content of extracellular fluid. Muscle cells are similarly impermeable to the bicarbonate anion. Smooth muscle contains twice as much chloride as striated muscle. The chloride level of skin and subcutaneous tissue is quite variable; these tissues normally store about one third of the chloride, but their chloride storage capacity increases remarkably during edematous conditions. The adult human contains only 210 mg. per cent chloride in his body water, in contrast to 270 mg. per cent in the fetus, and there is proportionately less extracellular fluid in the adult. The highest chloride levels are found in those body fluids which contain the least protein (cerebrospinal fluid, lymph, gastrointestinal secretions, and transudates). Desoxycorticosterone is necessary for normal retention of chloride in the extracellular fluids, as evidenced by low values in adrenal cortical insufficiency. When radioactive chloride (Cl^{38}) is injected into rabbits, it is transferred rapidly to cartilage, kidney, liver, and muscles, but not to the brain.

Normally, more than 90 per cent of the chloride is excreted in the urine, and most of the remainder in the feces and sweat. The urine of an adult contains about 7 gm. chloride daily; this anion is concentrated only slightly by the kidney (Table 96, page 572). Ingested table salt is excreted largely within from four to five hours. The rate of the urinary chloride excretion depends on the chloride intake and the water balance. Retention occurs readily during edematous conditions; three weeks may be required to establish chloride balance in nephrosis. Starvation, fever, vomiting, diarrhea, and excessive sweating also lower the urinary chloride output. When the plasma chloride level is slightly subnormal (*i.e.*, below a threshold value of 335 mg. per cent), the urinary excretion of chloride is markedly decreased, except in adrenal cortical insufficiency. Deficit of desoxycorticosterone increases the chloride output, by interfering with tubular reabsorption of this anion. Approximately twice the quantity of chloride present in the plasma is secreted daily in the gastric juice, and reabsorbed again; almost four times the total plasma chloride can be lost through severe vomiting.

POLYVALENT IONS

Calcium and the Metabolism of Bone

The calcium of biological fluids is determined by precipitation as oxalate and titration with permanganate; or it can be precipitated from trichloroacetic acid filtrates as the phosphate, and determined colorimetrically. The body contains more calcium than any other mineral; almost 1.1 kg. is present in the human adult.

The daily calcium requirement for growth and maintenance is 0.5 gm. total, or 8 mg. per kg., in the adult; 24 mg. per kg. in children, lactating

and pregnant women; and from 50 to 55 mg. per kg. in infants. Owing to partial loss of dietary calcium in the feces, the daily intake should be about 1 gm. for adults and children, 1.6 gm. for pregnant women, and more than 2 gm. during lactation. The average daily intake of the adult is slightly in excess of 1 gm. The quantity of this mineral which is retained increases with the calcium intake and with the vitamin D intake (daily optimum is 400 international units). Since calcium is deposited in tissues most rapidly during the last few months of fetal life, premature infants exhibit subnormal skeletal development, and they require special calcium and vitamin D therapy. Clinical syndromes due to calcium deficiency appear most rapidly in infants, children, and lactating women. Symptoms of deficiency in the young include stunted growth, weak muscles and osseous abnormalities. From five to ten years may be required for the development of serious deficiency in adults, and relatively long periods of dietary treatment are necessary to correct it. Foods with high calcium content are listed in Table 99, page 581. Milk and dairy products provide 80 per cent of the adult calcium intake; the calcium of these foods is better retained than that of cereals, nuts, or vegetables. As much as 200 mg. of calcium may be obtained daily from the intake of hard water. Infants absorb about one half the calcium of breast milk and a third of that in cow's milk. The latter contains four times as much calcium as breast milk; one half of this calcium is present as caseinate. Children utilize about 20 per cent, and human adults 30 per cent, of their ingested dietary calcium.

From 30 to 50 per cent of ingested calcium carbonate, chloride, gluconate, lactate, or phosphate is absorbed in the intestine. Ingestion of calcium and strontium isotopes shows approximately 30 per cent absorption of these ions in man. Calcium absorption is favored by slight acidity of the intestinal contents; it can be increased by the administration of citrate or tartrate buffer mixtures or by lactose, which stimulates bacterial production of lactic acid (page 154). Sufficient vitamin D and a limited fat intake are necessary for adequate absorption of calcium; vitamin D lowers the pH of intestinal contents and of feces. Bile salts assist calcium absorption by accelerating the absorption of fatty acids and preventing undue loss of insoluble calcium soaps in the feces. Dietary protein has a favorable influence on calcium absorption. Alkali ingestion, deficit of vitamin D, steatorrhea, obstructive jaundice, and an excessive intake of fat, fiber, phosphate, or oxalate (as in spinach) interfere with calcium absorption.

Calcium transport occurs in the blood. The erythrocytes do not contain calcium; to avoid errors from changes in corpuscular volume, serum is used for calcium analysis. The serum of normal adults contains 10.3 ± 0.3 mg. per cent calcium, and that of infants 11 mg. per cent. Forty-five per cent of this calcium is ionized, and 5 per cent exists as a citrate complex. The remaining 50 per cent consists of non-diffusible calcium-protein

complexes, whose concentration varies with that of the plasma proteins, especially the serum albumin (see embatic effect, page 56). The serum protein concentration constitutes an important regulating factor of the plasma calcium ion concentration:

$$[\text{Ca}^{++}] = K \frac{[\text{CaProteinate}]}{[\text{Protein}^{--}]}, \text{ where } K = 10^{-2.22}$$

The serum calcium ion concentration decreases as the plasma protein rises and as the hydrogen ion concentration falls (page 42). Increased calcium ion concentration depresses neuromuscular irritability, and, vice versa, extensive or rapid lowering of the plasma calcium ion concentration results in tetany, even though the calcium content of the brain is not changed. Rapid injection of ionizable calcium salt causes bradycardia and heart block. Calcium ions are coenzymic to thrombokinase and to choline esterase.

The ingestion of 2 gm. calcium chloride causes a rise of 1.5 mg. per cent in the serum calcium, by the second hour; the level returns to normal in four hours. Calcium gluconate causes a smaller, more prolonged rise. Administration of calcium salts with meals produces very little elevation of the plasma level. Intravenously injected calcium chloride leaves the blood stream rapidly. The serum calcium level is raised by injection of parathormone or of dihydrotachysterol (A.T. 10), administration of vitamin D or acidifying diuretics, and irradiation with ultraviolet light; it is also raised in such conditions as hyperparathyroidism, hyperthyroidism, and acidosis. Hypercalcemia occurs in birds during ovulation, and following injection of large doses of estradiol. Vitamin D not only promotes absorption of calcium; it also elevates low serum calcium levels in animals whose intestines are removed. It therefore aids in the maintenance of a normal serum calcium level. The chief regulator of plasma calcium concentration is parathormone. There is a maximal rise in serum calcium from twelve to eighteen hours after subcutaneous injection of the hormone, or from four to six hours after its intravenous administration. Serum calcium decreases markedly in hypoproteinemia and hypoparathyroidism (tetany), and to a slighter extent in pregnancy, old age, and conditions of deficient calcium absorption (vitamin D deficit, obstructive jaundice). Injection of phosphate or oxalate lowers serum calcium. Oxalate and citrate form calcium complexes and decrease the calcium ion concentration of plasma, even though citrate injections do not decrease the total serum calcium. Oxalate intoxication results in tetanic convulsions and death; citrate injection is relatively harmless, since the calcium-citrate complex is rapidly excreted by the kidneys.

Lymph, hepatic bile, and joint fluids have approximately the same calcium content as plasma; human milk has three times this concentration (Table 100, page 582). The low calcium levels of other body fluids are correlated with their small protein content. Virtually all the cal-

cium of cerebrospinal fluid is ionized; the level in cerebrospinal fluid is little influenced by administration of calcium or parathormone, or by parathyroidectomy. Intracellular calcium is more concentrated in nuclei than in cytoplasm. The kidney contains more calcium than do the other soft tissues; muscle has some calcium which has been shown by the electron microscope to reside chiefly in the anisotropic contraction bands. The calcium content of muscles increases in nutritional muscular dystrophy. Skin calcium decreases during childhood, and rises again in subsequent life; calcium is low in hyperplastic epidermis and in squamous cell carcinomas. The calcium concentration of brain is not readily altered, except in vitamin D deficiency, when it is lowered markedly without producing notable neurological symptoms. Parathormone has little effect on the brain calcium. The intravenous injection of vitamin D increases the calcium content of brain, heart, kidney, liver, thyroid, and adrenal glands.

The body skeleton is the main depository of calcium; it contains more than 90 per cent of the total body calcium. Experiments with radioactive calcium show that storage occurs in the bones, teeth, and skin. Radioactive Ca^{45} and Sr^{89} are transferred rapidly from the blood to the skeleton, especially to such areas of bone formation as the trabeculae. The bone ash contains 85 per cent calcium phosphate and 12 per cent calcium carbonate; the bone salt is an apatite, $n[\text{Ca}_3(\text{PO}_4)_2] \cdot \text{CaCO}_3$, in which $n = 2$ to 3. The carbonate component increases with age and during deficient phosphate intake; it decreases in acidosis and in fluoride poisoning. The phosphate component increases with low dietary calcium : phosphate ratios, and it is relatively low in newly deposited bone. Bone salt is deposited most rapidly during late fetal life, infancy, and childhood. In premature infants, adequate vitamin D administration shifts mineral from the bone shafts to the growing ends. Bone salt is mobilized readily throughout life; the trabecular and subepiphyseal bone constitute a particularly mobile mineral depot. Calcium is removed from bone during pregnancy and lactation. As much as one third of the skeletal calcium can be removed in three weeks by drastic acidosis or by the administration of thyroid or parathyroid hormones. Parathormone is a very active osteoclastic stimulant; the minerals removed from bone by the osteoclasts are subsequently redistributed to the soft tissues.

Growing zones of bone have a more extensive circulation and contain more osteoblasts than old cortical bone. During ossification, the inorganic phosphate is first converted to phosphoric esters of carbohydrate intermediates by the action of phosphorylases. Calcium is concentrated in cartilage by combination with the chondroitin-sulfuric acid prosthetic radicals of the cartilage chondroproteins. In the ossifying zones, it combines with the phosphate esters. The high calcium ion concentration of these regions can be demonstrated by staining with sodium alizarinate. The phosphate esters are hydrolyzed by the alkaline phosphatase of osteoid tissue. Hence, calcification is maximal near the provisional zone,

where the phosphatase concentration is high. Also, pathological calcification occurs most readily in those non-osteoid tissues which have a liberal phosphatase supply (the kidneys, gastro-intestinal tract, lungs, blood vessels, and mammary glands). The inorganic phosphate, liberated by the phosphatase of osteoblasts or chondroblasts, reacts with calcium to form colloidal calcium phosphate. The latter unites with other salts, through secondary valences, to form apatites of the general structure: $n\text{Ca}_3(\text{PO}_4)_2 \cdot \text{BX}$, in which $n = 2$ to 3 , $\text{B} = \text{Ca, Mg, Pb, or Ra}$, and $\text{X} = \text{CO}_3, (\text{OH})_2, \text{F}_2, \text{O}, \text{Cl}_2, \text{SO}_4, \text{ or P}$. The principal salt of normal bone is carbonate-apatite (dahllite). A smaller quantity of hydroxy-apatite is formed in bone by the action of water; this apatite preponderates in tooth enamel. The dustlike osseous deposit of apatite is not visibly crystalline, but x-ray spectrograms demonstrate its crystal lattice structure. The crystal lattices of bone salt are surrounded by a matrix of collagen fibrils. In the bone shafts, the lattices are arranged parallel to the length of the bone. Stresses produced by muscular contraction and body weight affect the bone architecture by influencing the orientation of the mineral lamellae.

Vitamin D generally assists the deposition of bone salt; but when a thousand times the customary dosage is administered, this vitamin acts like parathormone to cause withdrawal of bone calcium, transfer of the mineral from the shafts to the epiphyses, and hypercalcification of the growing zones of bones and teeth. Vitamin D and parathormone, the two important regulators of calcium metabolism, ordinarily have independent opposing effects on ossification. Any antagonism which they exhibit is mediated through the plasma calcium level, which is reciprocally related to the rate of parathormone secretion. Deficiency of vitamin D tends to lower serum calcium, and to stimulate the secretion of parathormone, with resulting maintenance of a nearly normal plasma calcium level. An osteoclastic reaction is established within six hours after the injection of parathormone. Repeated administration of the hormone results in the development of a refractory state, in which osteoclastic activity is inhibited. (See antihormones, page 679.)

While the calcium, phosphate and vitamin D intake and the rate of secretion of parathormone are important factors in ossification, certain accessory factors should be mentioned. Calcification usually requires that the product of the concentrations (in mg. per cent) of serum calcium and phosphorus be 30 or more. A relatively high tissue pH (7.4) is necessary for optimal phosphatase activity. The pH of cells in the actively ossifying zone is 7.5, while the cells of cartilage and connective tissue have a pH of approximately 7.2. Other stimulants of ossification influence the development of osteoid tissue. These include vitamins A and C, which allow normal cartilaginous and osteoblastic development, and thiamin, which is necessary for normal longitudinal growth of bone. Vitamins C and D also assist the formation of early dentine. Anterior pituitary growth

hormone stimulates the growth of cartilage; in excessive quantities this hormone produces arthropathy and hypertrophy of cartilage. The epiphyses remain open in juvenile hyperpituitarism and in castrates and eunuchoids, with a resulting overgrowth of the long bones. Thyroglobulin and large doses of androgens or estrogens induce epiphyseal union. Estrogens stimulate osteoblastic proliferation and activity, and they augment medullary proliferation of bone, except in large quantities, when they inhibit growth of cartilage; androgens tend to accelerate bone growth and to counteract the calcifying effects of estradiol (page 695). Normal bone growth requires an adequate supply of thyroid hormone; hypothyroidism in children causes osseous retardation, and in hyperthyroidism calcium is removed from bone very rapidly. The accumulation of injected porphyrins in actively ossifying areas of bone (trabeculae, epiphyseal plates and subperiosteal regions of growing animals, and the fracture callus of adult bone) suggests a possible physiological role for these pigments.

Calcium is excreted principally by the intestine and liver. Fecal excretion of calcium phosphate and calcium soaps continues on calcium-free diets and it is enhanced by alkalis, vitamin D deficiency, subnormal phosphate intake, high fat intake, steatorrhea, diarrhea, and hyperthyroidism. Very excessive doses of vitamin D cause diarrhea and calcium loss. Under normal conditions, the urinary output accounts for only 20 ± 10 per cent of the calcium output, or about 0.2 gm. daily in the adult. When the plasma calcium level falls below 7 mg. per cent, the urinary excretion of this mineral is markedly decreased. The kidneys excrete calcium more slowly than sodium, and they concentrate it approximately twofold. Negative calcium balance and diversion of calcium to the urine result from acid diets, acidosis, hyperthyroidism, hyperparathyroidism, excessive vitamin D administration, and low calcium diets. Types of acidosis which provoke the excretion of anions other than phosphate cause marked increase in the urinary calcium output, whereas phosphaturia tends to increase the excretion of sodium and potassium.

Magnesium

This mineral is determined in biological material, after deproteinization with trichloroacetic acid and removal of calcium oxalate, by precipitating magnesium ammonium phosphate and determining the latter colorimetrically. Magnesium is a dietary essential; in echinoderm embryos it is necessary for the formation of the skeleton and stomach. In rats, experimental magnesium deficiency causes excessive calcification of bone, pathological calcification of the kidneys, heart, and arteries, as well as dermatitis, hepatitis, vasodilation, and convulsions (low magnesium tetany with vasomotor spasm). The daily magnesium requirement of young children has been estimated as 12 mg. per kg. of body weight. As little as 0.22 gm. per day is sufficient for the normal adult, but lactating women

do not preserve their magnesium balance with three times this intake. Since the average daily magnesium intake of the human is approximately 0.4 gm., clinical magnesium deficiency is very rare. Cereals and nuts contain more magnesium than do other common foods (Table 99, page 581), but their magnesium is less readily absorbed than that of milk and green vegetables. In green plant tissues, magnesium is found as an integral portion of the chlorophyll molecule (page 528) from which it is split by gastric and intestinal secretions. The intestinal absorption of magnesium is less complete than that of calcium, and it is less intimately dependent on vitamin D. In other respects, the absorption of the two cations is regulated by the same factors. The inorganic salts of magnesium tend to act as cathartics, especially when the anion is polyvalent. Excessive intake of magnesium causes deficient absorption of calcium and phosphate.

Normal serum contains 2.4 ± 0.7 mg. per cent magnesium, approximately 85 per cent of which is diffusible; the erythrocytes contain about 6.6 mg. per cent (Table 100, page 582). In young animals, the erythrocytic magnesium concentration is higher than in adults. Serum magnesium is increased by the injection of calcium salts or parathormone. Owing to the small fraction (15 per cent) which is present as magnesium-protein complex, the serum magnesium level is not affected greatly by variations in the plasma protein level. Slight elevations of serum magnesium can cause hyperglycemia and glycosuria. By injecting 0.1 or 0.2 gm. magnesium salt per kg. of body weight, the serum level can be elevated above 5 mg. per cent, with production of profound narcosis, low body temperature, and artificial hibernation in animals. Larger injections are fatal; a serum level of 17 mg. per cent causes coma. Injections of magnesium salts are, at times, used to produce an antispasmodic effect. Low serum magnesium has been reported in certain patients who have mild tetany (page 631).

The magnesium concentration is high in milk and low in bile, while the level in other body fluids is comparable to that in plasma (Table 100, page 582). The magnesium concentration of cerebrospinal fluid is rather independent of the plasma level. Soft tissues contain from 11 to 21 mg. per cent, with the exception of the intestine, lungs, skin, and certain endocrine glands, in which lower concentrations are found. During growth, the magnesium content of the skin parallels that of calcium. The skin magnesium is decreased in chronic disseminated neurodermatitis. The injection of narcotic doses of magnesium sulfate does not produce any change in the calcium or magnesium content, or in oxygen consumption of the brain, but it increases adenosine triphosphate in muscles. The bony skeleton contains one half the body magnesium, and the muscles one third. By means of the electron microscope, it has been shown that the magnesium in striated muscle is localized in the anisotropic contraction bands. Intracellular magnesium is more concentrated in nuclei than in

cytoplasm. Magnesium ions are coenzymic to leucylpeptidase, phosphatases, and phosphorylases (page 597). Carboxylase is a diphosphothiamin-magnesium-protein compound, which contains 0.13 per cent magnesium (1 gm. atom per mol). Enolase, glucophosphomutase, and phosphorylase are complexes of magnesium and proteins. The skeleton contains only 1 per cent as much magnesium as calcium; the magnesium content of bone tends to vary inversely with the calcium concentration. Magnesium deficiency increases the calcium content of arteries, heart, kidney, and muscle, and causes renal degeneration with nephrotic symptoms.

The feces contain 65 ± 15 per cent of the normal magnesium output. Intravenously injected magnesium salts are excreted principally in the urine, together with extra calcium. The kidney normally concentrates magnesium more than sodium, chloride or calcium. Acidifying measures and acidosis cause less increase in urinary magnesium than in urinary calcium. The administration of high phosphate diets and thyroid and parathyroid hormones has little influence on the magnesium excretion. Ingested inorganic magnesium salts act as acidifying diuretics, while the hydroxide and carbonate are alkalinizing agents.

Phosphate

The inorganic phosphate of biological material can be determined colorimetrically in trichloroacetic acid filtrates, by forming phosphomolybdic acid and reducing the latter to a blue colored compound. Phosphate esters are hydrolyzed by digesting with acid, and the resulting inorganic phosphate is determined as above. Adenosine triphosphate, adenosine diphosphate, phosphoglycerate, and inorganic phosphate can be separated from other phosphate esters by the insolubility of their barium salts. The total quantity of phosphorus in the body is second only to calcium (Table 100, page 582). Phosphate is present in all cells, body fluids, and natural foods, chiefly as inorganic orthophosphate and as esters of carbohydrates, lipides, proteins, and the like. The intimate relations of phosphate to the metabolism of these foods have been discussed in preceding chapters.

Phosphate deficiency causes muscular weakness and limitation of growth and of ossification. In cattle, failure of estrus and milk secretion and the development of a depraved appetite for bones and feces (osteophagia and coprophagia) result from phosphate deficiency. Phosphate retention increases with the intake, and with the vitamin D dosage. Infants require from 45 to 50 mg. phosphorus per kg. of body weight, and 350 international units of vitamin D daily; the adult requires about 13 mg. phosphorus per kg. of body weight. The average phosphorus intake of the adult is about twice this quantity (1.7 gm. daily). Safe daily allowances of phosphorus in the forms found in cow's milk are 95 mg. per kg. for

infants, 1.3 gm. total for children and normal adults, and 2.6 gm. for lactating women. Only inorganic phosphate is necessary in the diet. Common high phosphate foods are listed in Table 99, page 581; a large fraction of the phosphate of cereals, nuts, certain legumes, and chocolate is present in unavailable form (phytin, page 280). This inositol ester precipitates calcium, is digested with difficulty, and the small quantity which is absorbed is rapidly excreted by the intestine. When the phytin-containing foods are the sole source of phosphate, mammals develop rachitis. Other phosphate esters of foods are readily hydrolyzed by the phosphatases of the bile, pancreatic juice, succus entericus, and intestinal mucosa. Some of the dietary phosphate of humans is derived from cereals, meat and fish, but the best sources are dairy products. Ideal phosphate foods for infants have a calcium : phosphate ratio near 2; the optimal ratio for adults is approximately 1. In breast milk the ratio is 2.25, but in cow's milk it is 1.35. In general, the factors which control calcium absorption and balance also apply to phosphate. Vitamin D is especially important for phosphate absorption, largely because of its beneficial effect on calcium absorption. Studies with radioactive phosphorus (P^{32}) show that about two thirds of ingested inorganic phosphate is absorbed in eight hours, and that the absorption is accelerated by dietary glucose and is decreased by iron salts.

The distribution of the various blood phosphate fractions is given in Table 100, page 582. The sum of the inorganic and ester phosphorus is termed the *acid soluble phosphorus*. The serum of adult humans contains 3.7 ± 0.8 mg. per cent inorganic phosphorus and 1 mg. per cent ester phosphorus. The plasma and erythrocytes of young mammals have relatively high phosphorus concentrations. The erythrocytes contain only phosphate esters; lipid phosphate is present in both portions of blood. The principal phosphate ester of the erythrocytes is diphosphoglyceric acid, which is present to the extent of 130 mg. per cent in adult erythrocytes; this fraction is high in early childhood (Table 100). It decreases in acidosis and rachitis, and increases after pyloric obstruction and after the injection of insulin or parathormone. The blood levels of diphosphoglycerate and chloride often exhibit inverse reciprocal variations. Below pH 7.3 the erythrocytic diphosphoglycerate is rapidly decomposed to inorganic phosphate; the concentration of this substance appears to be related to the labile phosphorus reserve of the body. Other phosphate esters of human erythrocytes include 95 mg. per cent of adenosine triphosphate, 4 mg. per cent of cozymase II, and 2 mg. per cent of cozymase I. Avian and turtle erythrocytes contain considerable phytin. Reticulo-cytes have more phosphorus than adult erythrocytes, owing to excess adenosine triphosphate and the presence of some nucleic acid. The inorganic phosphate of serum is of considerable importance in clinical studies; it is increased by exercise, ultraviolet irradiation, and administration of thyroid hormone, dihydrotachysterol (A.T. 10), and vitamin D. The latter tends to maintain the normal serum inorganic phosphate level,

which, therefore, varies with the season and the diet. Serum inorganic phosphate is lowered slightly by injection of insulin and ingestion of carbohydrate, and markedly by vitamin D deficiency and the injection of parathormone. Adrenaline causes an initial decrease in the serum inorganic phosphate, followed by an increase. The inorganic phosphate of serum often varies inversely with the serum calcium; it is usually lowered by intravenous injections of calcium salts, and vice versa, a large unbalanced phosphate intake can cause tetany.

Experiments with radioactive phosphorus show that the phosphate anion remains in the body of a rabbit for about thirty days; during this time it undergoes esterification to form various esters, and is exchanged with tissue inorganic phosphate. The cellular concentrations of the latter are more dependent on carbohydrate metabolic processes than on the Donnan equilibrium. Inorganic radioactive phosphate is incorporated into the acid soluble esters, phospholipides, and so forth; the terminal phosphate radical of adenosine triphosphate is exchanged very rapidly, and erythrocytic diphosphoglycerate is renewed at a fast rate. Phosphate passes into the cerebrospinal fluid more slowly than do monovalent ions; it is transformed into esters in the choroid plexus. Some of the absorbed radioactive phosphate is transferred to muscles, and another portion to the liver, intestine, lungs, and kidneys, where it is esterified rapidly. The brain and teeth are least active in phosphate exchange. Phospholipides and nucleoproteins of normal tissues exchange their phosphate less rapidly than do the acid soluble esters; the nucleoproteins of tumor nuclei and of regenerating tissues show more active exchange. Eventually, most of the absorbed phosphate is deposited in the bones.

About 80 per cent of the total body phosphate is in the skeleton, and 10 per cent in the muscles (Table 100, page 582). In most tissues, about one half the phosphate is present as nucleic acid prosthetic radicals. In the liver, it is distributed approximately equally as acid soluble phosphate, phospholipide and nucleoprotein, while in the brain, about 75 per cent is present as phospholipide and only 5 per cent as nucleoprotein. Acid soluble phosphate constitutes 75 per cent of the total phosphate of striated muscle, and 40 per cent of that in smooth muscle. The muscle and liver phosphates are closely related to carbohydrate metabolism. Small quantities of inorganic phosphate are withdrawn from the plasma and transferred to these tissues during active sugar utilization. The hepatic acid soluble ester phosphate is lowered by prolonged high fat diets, and elevated by a liberal carbohydrate intake or by the injection of insulin. As shown by radioactive phosphate, insulin increases the turnover rate of adenosine triphosphate and acid soluble phosphorus in the liver, and of phosphocreatine and adenosine triphosphate in muscle. Stimulation, or injury, of nerve and muscle frees phosphate anions from the acid soluble esters and produces a condition of electronegativity. The bone phosphate is a mobile store, subject to the various mobilizing factors discussed on

page 590. Studies with radioactive phosphate show that 30 per cent of the bone phosphate can be exchanged within three weeks. Phosphate of epiphysial bone is more labile than that in diaphysial bone. The chief factors in deposition of bone phosphate are the intake of calcium, phosphate, and vitamin D. Acidifying measures, parathormone, and thyroid hormone effect its withdrawal. Radioactive phosphate tends to accumulate as acid soluble esters in the epiphyses of rachitic bone, indicating a defect in the metabolism of these esters. Calcification of cartilage *in vitro*, and the activity of the phosphorylases concerned in the formation of phosphate esters, are inhibited by phlorhizin and iodoacetate. The calcifying activity can be restored after phlorhizin inhibition by adding glucose-1-phosphate.

About 40 ± 10 per cent of the phosphate is normally excreted in the feces, and the remainder appears in the urine. Approximately one half the fecal phosphate is inorganic, 30 per cent is in proteins, 20 per cent in phospholipides and 2 per cent in phytin. The intestinal excretion of phosphate is diminished by high fat diets, and by the administration of vitamin D; it is increased by ingested alkali, or by diets containing considerable calcium or magnesium. The urinary phosphate excretion (about 1 gm. daily in the normal adult) is important in acid-base balance (page 33). Urinary phosphate is estimated to average 1.2 equivalents per mol, in contrast to 3.0 equivalents for the bone and fecal phosphate. The kidney concentrates inorganic phosphate about twenty-five times. Tubular reabsorption of phosphate in man is in the vicinity of 3.8 mg. of phosphorus per minute per 100 ml. of glomerular filtrate. Maximal reabsorption of phosphate is increased by vitamin D and decreased by acidosis and by parathormone. Only 5 per cent of the urine phosphate is normally present in ester form. The latter includes only 1 mg. of glycerophosphate per day; more glycerophosphate is excreted during starvation and after morphine administration. The phosphate output derived from protein catabolism is equal to 5.75 per cent of the urinary nitrogen. Phosphate and calcium are diverted to urine under the same influences (page 591), with the exception of high fat diets. Urinary phosphate is increased by acidosis, starvation, and leukemia, and by the administration of thyroid hormone, parathormone, vitamin D, or dihydrotachysterol (A.T. 10).

Other Phosphorus Anions. Hypophosphite (H_2PO_2^-) is absorbed and excreted unchanged. Phosphite (HPO_3^{--}) and metaphosphate (PO_3^-) are oxidized by animals to orthophosphate. Pyrophosphate ($\text{H}_2\text{P}_2\text{O}_7^{--}$) is partly hydrolyzed to orthophosphate in the acid gastric contents; it is also absorbed partly and excreted in unchanged form. Pyrophosphates are present in certain acid soluble esters of tissues (adenosine triphosphate, cocarboxylase, cozymases, flavin nucleotides). The pyrophosphate esters are hydrolyzed by pyrophosphatase and by alkali. *Pyrophosphatase*, with an optimum pH zone of 7.8 to 8.2, is found in numerous tissues; it is activated by magnesium ions and inhibited by calcium ions. The blood pyrophos-

phatase is present in the erythrocytes. Myosin is an *adenylpyrophosphatase* which hydrolyzes adenosine triphosphate to adenosine diphosphate; it can also hydrolyze inosine pyrophosphate and triphosphate. This enzyme is activated by calcium ions and inhibited by cupric, magnesium, and fluoride ions. Other adenylpyrophosphatases occur in the liver and kidney.

Phosphatases. The non-specific phosphatases, or phosphomonomesterases, are enzymes which hydrolyze such monophosphoric esters as glycerophosphates, ribose- and hexose-monophosphates, phenyl phosphate, phosphocreatine, phosphoserine, and mononucleotides. Two general types are recognized, namely, "acid" and "alkaline" phosphatases, with optimal pH zones at 5 to 6 and 8.5 to 9.5, respectively. The acid phosphatase of the kidney is a water soluble protein, and the alkaline phosphatase is a globulin. Tissues also contain certain specific phosphatases, including choline phosphatase, ribonuclease, thymonucleodepolymerase, and 5-nucleotidase which hydrolyzes muscle adenylic acid. Magnesium and manganous cations are coenzymic to alkaline phosphatase. Phosphatases are inhibited by fluoride, bile salts, and cardiac glycosides; the inhibitory effect of fluoride is not entirely specific. *Phosphorylases*, which are concerned with transphosphorylation and are widely distributed in animal and plant tissues, are distinct from the phosphatases. Muscle phosphorylase has been crystallized as a euglobulin-adenylic acid-magnesium complex; approximately 60 mg. per cent of this enzyme is present in rabbit muscle. Phosphorylases are activated by magnesium, and inhibited by phlorhizin and iodoacetate. Cyanide interferes with phosphorylation by inhibition of the respiratory reactions which provide the necessary energy. The proximal renal tubule, which exhibits marked phosphatase and phosphorylase activities, is the site of phlorhizin action on the kidney. (See page 318 for a discussion of phosphorylation.)

The non-specific phosphatases are found in all living cells. Both types occur in cytoplasm, while the nuclei seem to contain only alkaline phosphatase. Mammalian tissues usually contain both the acid and alkaline types; erythrocytes, the prostate, and plant tissues have only the acid type, which is not activated by magnesium cations. Acid phosphatase is prominent in the prostate, gastric mucosa, liver, muscle, skin, and spleen. Alkaline phosphatase is of considerable interest in medicine, since it represents the typical osteoid phosphatase produced by osteoblasts and chondroblasts. This enzyme is present in largest quantities in the intestinal mucosa, renal cortex, growing zones of bones, teeth, and hypertrophic cartilage, but it is also found in the adrenal glands, bile, blood vessels, central nervous system, intestinal secretions, liver, lungs, mammary gland, pancreatic juice, plasma, pancreas, spleen, and thyroid. Epiphyseal phosphatase activity in rats is decreased by injection of corticosterone and compound E, and it is increased by the injection of testosterone, estradiol or thyroxine. Phosphatase activity is low in scorbutic bone, gastric and intestinal adenocarcinomas, and mammary carcinomas; it is

high in hepatomas and meningiomas, and it increases in polymorphonuclear leukocytes during early stages of wound healing.

Normal plasma of adults and young children has alkaline phosphatase activities equivalent to 2.7 ± 1.2 and 9 ± 4 Bodansky units per 100 ml., respectively. A Bodansky unit is equivalent to the liberation of 1 mg. of inorganic phosphorus from a standard β -glycerophosphate solution in 1 hour at 37°C . and pH 8.6. The ingestion of unsaturated fatty acids raises the decreased phosphatase activity of the plasma and intestine in fasted rats. Plasma or serum phosphatase activity increases in vitamin D deficit, viosterol poisoning, phlorhizin diabetes, after parathormone injection, following pancreatectomy or hepatectomy, and in pregnancy, obstructive jaundice, and the bone diseases listed on page 626. The acid phosphatase activity of blood is normally equivalent to 1.5 ± 1.0 units per 100 ml. when measured at pH 4.9; it rises in the presence of prostatic tumors. The recent introduction of sodium phenolphthalein phosphate or sodium *p*-nitrophenyl phosphate as substrate for phosphatase determinations provides a rapid colorimetric procedure.

Bone phosphatase is closely related to osteoblastic activity (pages 589 and 596); it appears in fetal osteoid tissue when ossification begins. Metastasis of osteoblastic tumors causes an increase in the acid phosphatase of neighboring non-osteoid tissue. When bladder epithelium is transplanted to the sheath of the rectus abdominalis muscle, heterotopic bone is formed in the surrounding connective tissue. This phenomenon coincides with the local appearance of phosphatase. Repair of urinary bladder defects by connective tissue causes phosphatase generation, and bladder epithelium can be used as a bridge to stimulate bony union of non-uniting fractures. Similar transformations of connective tissue into osseous tissue have been reported for many mammalian organs *in situ*. The chemical nature of the substance which stimulates differentiation of fibroblasts into osteoblasts is unknown. Alcohol, ether and benzene extracts of osseous tissue contain the stimulatory substance.

The presence of alkaline phosphatase in non-ossifying tissues indicates that accessory factors are important for osseous activity. Magnesium and manganese act as coenzymes to alkaline or bone phosphatase, and vitamin D indirectly accelerates the action *in vivo*. Studies with radioactive phosphate show that lack of vitamin D does not prevent esterification of phosphate in bone, but the esters are not converted into bone salt at a normal rate. Manganese deficit in chicks and turkeys incites the disease known as perosis, which is characterized by shortening and thickening of bones and by the deformity termed "slipped tendon." Here, plasma and bone phosphatase activity is decreased, and administration of manganese cures the condition.¹ The normal biological transfer of phosphate from

¹ Choline deficiency in chicks produces a form of perosis in which the plasma and bone phosphatase levels are normal. Biotin deficiency can also produce perosis.

adenylic acid and cozymase is inhibited by excessive sodium concentration; this effect is counteracted by manganese or potassium.

Sulfate

Inorganic sulfate can be determined by precipitation as barium sulfate. In micromethods, the biological material is deproteinized with uranium acetate or trichloroacetic acid and the sulfate is precipitated as benzidine sulfate, which can be determined colorimetrically by treating it with sodium- β -naphthoquinone-4-sulfonate, or with hydrogen peroxide and ferric chloride. Etheral sulfate is hydrolyzed to inorganic sulfate by heating in acid solution. Neutral sulfur (organic sulfur) is oxidized to inorganic sulfate by heating with sodium peroxide or a mixture of copper nitrate and potassium chlorate.

Plants can utilize inorganic sulfate for growth and protein synthesis, whereas animals cannot. The most important dietary sulfur compounds are cysteine, cystine, methionine, biotin, and thiamin, whose sulfur constitutes 1 ± 0.7 per cent of the proteins of ordinary foods. The chief dietary sources of sulfur are such protein foods as meats, dairy products, and cereals. A protein intake which is sufficient to maintain nitrogen balance also provides adequate sulfur. The daily sulfur intake of the human adult is approximately 1.3 gm. Sulfur amino acids and inorganic sulfate are slowly absorbed in the intestine.

Human plasma contains 1.3 ± 0.5 mg. per cent of sulfur in the form of inorganic sulfate, and 2.7 mg. per cent as conjugated or etheral sulfate (Table 100, page 582). Plasma inorganic sulfate does not undergo marked increase when sulfates are administered orally. The inorganic sulfur of plasma rises to as much as 35 or 40 mg. per cent in severe renal impairment. Injected inorganic sulfate is partly retained by the liver and converted to etheral sulfate; this organ has a higher inorganic sulfate concentration than other tissues (Table 100). Tissue sulfur is largely organic in nature. For example, 70 per cent of the brain sulfur is protein sulfur; 23 per cent is lipide sulfur, and 7 per cent is inorganic sulfur. In addition to the sulfur amino acid units of proteins, the organic sulfur of tissues includes small quantities of ergothionine, polyuronide-sulfuric acids, sulfatides, taurine, thiamin, and so forth. The metabolism of these organic sulfur compounds has been considered on pages 436 to 439. Retention and storage of sulfur in tissues is interlinked with protein metabolism; it has little relation to water metabolism. About one half of orally administered radioactive inorganic sulfate is retained by the body, and exchanged with tissue sulfates. Injected S^{36} is assimilated most readily by the bone marrow.

Only one sixth of the normal sulfur excretion is performed by the intestine. The remainder is largely excreted by the kidneys, although some sulfur is lost as keratin in desquamated skin, hair, and nails. Absorbed inorganic sulfate, or sulfide (which is rapidly oxidized to sulfate in the

body), is excreted principally in the urine. Calcium, magnesium, and ammonium sulfates act as acidifying diuretics. The urine of an adult contains about 1 gm. of total sulfur daily, 0.8 gm. of which represents inorganic sulfate. The kidney concentrates the latter approximately fifty times. About 90 per cent of the sulfate in the glomerular filtrate is ordinarily reabsorbed in the renal tubules. The relations of inorganic sulfur to the other urinary sulfur fractions have been reviewed on page 438.

INTERRELATIONS OF THE PREDOMINANT MINERALS

General

Osmotic pressure and acid-base equilibria involve all the predominant minerals, but these substances are not freely interchangeable in living organisms. Muscle loses its excitability in solutions of non-electrolytes, and isotonic solutions of single salts are toxic to living cells, owing to their effects on cell permeability, irritability, contractility, and metabolism. Sodium is the least toxic of singly administered cations. The normal regulation of metabolic processes in tissues requires a definitely balanced mixture of inorganic ions. Ringer-Locke solution and 0.9 per cent sodium chloride solution are both isotonic to extracellular fluid of mammals, but the former contains physiologically balanced concentrations of certain predominant minerals. It consists of 0.92 per cent sodium chloride, 0.042 per cent potassium chloride, 0.018 per cent calcium chloride (anhydrous), 0.015 per cent sodium carbonate, and 0.1 per cent glucose. If any one cation is removed from Ringer-Locke solution and the modified solution is perfused through the heart, this organ gradually ceases to contract.

The predominant minerals consist of two physiologically antagonistic groups, namely, the univalent and the divalent ions. The ratio, sodium + potassium : calcium + magnesium, for example, is related to neuromuscular irritability.

Monovalent Ions

The sum of sodium and potassium is related directly to the volume of body fluid. The total concentration of these cations remains relatively constant in the extracellular fluid, and also in mammalian erythrocytes, even though the individual sodium and potassium concentrations vary reciprocally in the erythrocytes of different species. The ratio of sodium to potassium, which is correlated with the ratio of extracellular to intracellular water, is approximately 19 ± 4 in the extracellular fluids, 3 in the skeleton and skin, 1.5 in lungs, 0.9 in liver, 0.5 in brain, and 0.25 in muscles (calculated from values in Table 100, page 582). Neuromuscular irritability and tissue glycogenesis are depressed by a marked lowering of the sodium : potassium ratio in the extracellular fluid. The sodium : potassium ratio of plasma falls in Addison's disease, and at the onset of malarial chills; it increases periodically in familial periodic paraly-

sis. During clinical improvement of adrenal cortical insufficiency, the low ratios in plasma and muscle return to normal. The action of potassium cations on nerve and muscle cells is related to participation of these ions in metabolic processes, which provide the energy for muscular contraction and nerve conduction. Limited quantities of rubidium and caesium may be substituted for potassium, and lithium for sodium. These extraneous cations are more toxic than either sodium or potassium; rubidium is a more powerful nerve stimulant than potassium.

The sodium : potassium ratio in the average human diet is 1.7. The optimal ratio is much lower for young animals, owing to selective retention of potassium by the growing tissues; cow's milk, which has a ratio of 0.4, meets this requirement. Since vegetarians and herbivorous animals ingest food with a very low sodium : potassium ratio (Table 99, page 581), they develop an appetite for sodium chloride. Ingestion of either sodium or potassium causes increased urinary excretion of the other in adrenalectomized animals, and also in normal animals when a sufficient excess of either cation is administered. The body normally maintains its physiological ionic balances by excretory mechanisms; for example, the kidney can retain either ion of ingested table salt and excrete the other.

The sum of the monovalent anions, bicarbonate and chloride, is maintained constant in body fluids, despite reciprocal variations in the concentrations of the individual anions. The ratio of these anions fluctuates in disturbances of acid-base metabolism (page 33). It diminishes progressively in the gastro-intestinal secretions from the stomach to the colon. Bromide can be substituted rather successfully for chloride in Ringer-Locke solution and in body fluids, but it has a specific narcotic effect which is not shared by chloride. Bromide poisoning can be treated successfully by displacing this anion with chloride.

Polyvalent Ions

Interrelations of calcium and magnesium are physiologically important, since the calcium : magnesium ratio affects neuromuscular irritability. Injections of magnesium salts are, at times, used in the treatment of convulsive diseases; an increased plasma magnesium concentration paralyzes nerve endings and produces narcotic effects that may be counteracted by injections of calcium or potassium salts. Calcium excess causes rigor of muscles and depression of nervous irritability, but changes in the plasma calcium level (tetany) do not easily affect the calcium content of the brain, or its oxygen consumption. Rachitis is accompanied by a marked decrease in the brain calcium content without significant psychic or somatic effects. Low calcium or magnesium in plasma leads to tetany, but when both are decreased, tetany is ameliorated. Also, in puerperal paralysis the serum calcium is low and the magnesium high, and tetany does not occur. Both calcium and magnesium antagonize the neuromuscular depression and

stimulation of ganglia caused by excess potassium. Calcium or phosphate deficiency lowers muscle potassium and raises muscle sodium.

The calcium : magnesium ratio is 100 or more in bone, 1.8 to 4 in body fluids, cartilage, lungs, and skin, and 0.3 to 0.7 in brain, liver, muscle, and spleen. A high dietary intake of calcium intensifies the effects of magnesium deficiency, and vice versa. The two minerals exert inverse effects on ossification; the calcium content in rachitic bone is low, and its magnesium content is high. Excess magnesium tends to hinder normal ossification, and to provoke the formation of urinary calculi. The injection of magnesium salts increases the excretion of calcium in the urine.

Some of the antagonisms exhibited by calcium and magnesium are traceable to their competition for phosphate. The dietary calcium : phosphorus ratio should ordinarily approximate 1.3, the ratio found in cow's milk. Marked imbalance of dietary calcium and phosphate causes deficient retention of both minerals. The ratio of the retained minerals is from 1.5 to 2 in infancy, from 1 to 1.5 in childhood, and less than 1 in the adult. Ratios below 0.33 cause decalcification; cereals have low ratios, and cereal diets are rachitogenic. A high dietary calcium : phosphorus ratio assists in the control of tetanic symptoms in parathyroidectomized animals. Vitamin D functions to preserve a normal calcium : phosphorus ratio in the serum. The product of serum calcium and inorganic phosphorus concentrations tends to remain constant in normal individuals; hence, the injection of either calcium or phosphate lowers the plasma concentration of the other.

The interrelations of predominant divalent anions have received little study, but it is known that an antagonism exists between monovalent and divalent anions. The chloride : phosphorus ratio is very low in muscle, brain, and bone, as compared with that in extracellular fluids and cartilage. The chloride : phosphorus ratio is related to the distribution of fluid. Phosphate and bicarbonate anions are associated with intracellular energy-yielding metabolic processes. Borate can substitute partially for physiological anions in the antagonism of chloride.

The complex biological activities and interrelations of inorganic ions are usually interpreted as physical effects on tissue colloids (in terms of the Hofmeister series). However, in the dilutions encountered in tissues, the dehydrating properties of the several ions are of less consequence than their individual chemical activities. Ionic effects on colloids are significant for cell permeability, but the specific chemical properties of ions affect the metabolic processes of cells. Individual inorganic ions act as specific coenzymes and enzyme inhibitors *in vivo*. Copper, iron, potassium, sodium, and bicarbonate ions have been shown to be active salt catalysts for carbohydrate oxidation by hydrogen peroxide *in vitro*, whereas calcium and chloride ions do not share this effect. Similarly, tissue respiration in the Warburg apparatus is increased by copper, iron, magnesium, potas-

sium, sodium, bicarbonate, borate, phosphate, and sulfate ions. while calcium and chloride ions inhibit it.

METABOLISM OF THE TRACE MINERALS

"Discovery of truth is the sole purpose of philosophy, which is the most ancient occupation of the human mind and has a fair prospect of existing with increasing activity to the end of time." — AMBROSE BIERCE

Iron, copper, cobalt, manganese, zinc, and iodide are dietary essentials of mammals; other trace minerals found in tissues have not been proved necessary for mammalian life.

ESSENTIAL TRACE MINERALS

Iron

In the determination of inorganic iron, the metallic cation is reduced to the ferrous state prior to deproteinization of the biological material with trichloroacetic acid; the ferrous iron can then be determined colorimetrically by means of α , α' -dipyridyl. The determination of organic iron requires ashing; the total iron is then estimated colorimetrically, by dipyridyl if in the ferrous state, or by thiocyanate if in the ferric state.

Meat juices, liver, green leafy vegetables, molasses, egg yolk, and meats have high iron concentrations (Table 101). Milk is a poor source of iron, although breast milk contains slightly more than cow's milk (Table 102). The iron content of milk is not increased appreciably by administration of iron salts to lactating animals. The ferrous cation is absorbed by the stomach, and especially by the small intestine; ferric salts are reduced to ferrous cations by organic components of the chyme, prior to absorption. The hydrochloric acid of gastric juice, and certain organic acids of fruits, facilitate the solution of insoluble iron compounds and accelerate the gastro-intestinal reduction of iron. Ferrous hydroxide is soluble below pH 5, whereas ferric hydroxide precipitates above pH 2.5. Hence, soluble ferrous salts can be absorbed by fundusectomized dogs; achlorhydria hinders the utilization of less soluble iron compounds. Most of the iron in meat, liver, molasses, egg yolk, cereals, cocoa, legumes, and yeast is physiologically available for absorption, while less than one half of that in nuts, berries, spinach, and certain other leafy vegetables can be utilized. Ferrocyanides are absorbed poorly; the iron of ingested hemoglobin is unavailable, since it is not easily liberated from hematin in the digestive tract. Dietary phytic acid depresses iron absorption. For therapeutic purposes, iron is administered by mouth as ionizable ferrous or ferric salts in divided doses not to exceed 2 gm. daily. Larger dosage tends to irritate the intestine and to impair calcium and phosphate absorption by precipitating insoluble iron phosphate. Long-continued

TABLE 101

COMMON FOODS WITH HIGH CONCENTRATIONS
OF TRACE MINERALS

(In Mg. Per Cent)

IRON		COPPER		MANGANESE	
Beef juice	45	Calf liver	4.4	Wheat bran	9
Hog liver	25	Oysters	3.1	Blueberries, huckle- berries	4.4
Parsley	19	Chocolate	2.7	Pecans, wheat	3.5
Beef liver, molasses	8	Beef liver	2.2	Chocolate, graham bread	3.1
Egg yolk, sweetbreads	7.5	Molasses, mushrooms	1.9	Oatmeal	2.8
Raisins, watercress	7	Pecans	1.4	Shredded wheat	2.4
Avocado, bran flakes	6	Peanuts, walnuts	1.0	Walnuts	1.8
Dates	5	Pickles	0.8	Barley, peanuts	1.5
Wheat	4.5	Lobsters, wheat	0.7	Cocoanut, rye bread, beets	1.3
Beef, oatmeal, pickles, spinach	4	Rice	0.6	Pineapple, polished rice	1.1
Chocolate, maple syrup, mushrooms, oysters, reinforced foods (bread, breakfast cereal, or flour)	3	Bran flakes, olives	0.5	Bananas, lettuce, pars- ley	0.9
Leafy vegetables	0.4-19	Nuts, cereals	0.06-1.4	Fruits	0.02-4.4
Nuts, cereals	1-8	Fruits, tubers, leafy vegetables	0.01-0.3	Nuts, cereals	0.6-3.5
Meats	1.5-4.0	Meat, fish	0.01-0.1	Leafy vegetables, tubers	0.05-1.3
Tubers, fruits	0.25-2.5			Meat, fish	0.01-0.06
Fish	0.8-1.7				
IODINE ^{1,2}		ZINC			
Cod liver oil	770	Oysters	40		
Sea foods	20-260	Commercial casein	30		
Agar	165	Cereals, fruits, meat, vegetables	0.05-5		
Bacon, eggs, oats, peaches	16	Cow's milk	0.4		
Butter, lamb	15				
Apple sauce, bread, celery	13				
Asparagus, bananas	11				
Beef	9				
Chocolate beverage, lemons, olive oil, canned peas, potatoes	6.5				
Cottage cheese, cream, spinach	6				
Corn, fresh water fish	5				

¹ In γ per cent.² Varies with the locality.

intake of excess iron salt initiates a form of rachitis intractable to vitamin D. Mixing iron salts with foods causes gradual oxidation of vitamins A and E. The absorption of iron is slow and incomplete; in dogs, 66 per cent of a 1 mg. dose of radioactive iron cation is absorbed, but only 7 per cent of an 84 mg. dose. Excess dietary phosphate, increased gastrointestinal activity and loss of bile through fistulae inhibit iron absorption. Dogs with experimental anemia exhibit an increased rate of iron absorption. Studies with radioactive iron indicate that pregnant women and patients with iron deficiency anemias have an increased ability to absorb

iron. Increased iron absorption is initiated about one week after acute hemorrhage. The recommended daily intake of this mineral is: at least 1 mg. per kg. of body weight for the artificially fed infant, 0.6 mg. per kg. for young children, 0.2 mg. per kg. (or 12 mg. total) for the normal adult, and 0.3 mg. per kg. (or 20 mg. total) for the pregnant woman. Inadequate iron intake leads to hypochromic anemia, especially in infancy and childhood, and in females during adolescence, pregnancy, and menopause.

The normal total iron concentration of the blood is 50 ± 10 mg. per cent in men, and 44 ± 2 mg. per cent in women. Over 98 per cent of this iron is present in the hemoglobin. The ratio, blood iron (mg. per cent): erythrocyte count (in millions), is known as the iron index; it is 9.2 in normal adults. (Calculated from Table 94, page 544, and Table 102.) The serum iron is normally 0.13 ± 0.05 mg. per cent in men, and 0.10 ± 0.04 mg. per cent in women (Table 102). The absorbed ferrous cations are quickly oxidized by tissues and blood, and transformed to a ferric-globulin complex which represents the serum transport form of this mineral. Iron is thus transported as a complex

TABLE 102
APPROXIMATE TISSUE CONCENTRATION
OF TRACE MINERALS

	MG. PER CENT				γ PER CENT			
	Iron	Copper	Zinc	Fluorine	Cobalt	Nickel	Manganese	Iodine
Blood ¹	$50 \pm 10^*$	0.12 ± 0.03	0.6 ± 0.1	0.05 ± 0.03			15	$8.5 \pm 3.5^*$
Serum	$0.13 \pm 0.05^*$	0.11 ± 0.03	0.4					
Erythrocytes	110 ± 20	0.14	0.8					
Milk								
Cow	0.04	0.03 ± 0.02	0.4	0.15				
Human	0.2	0.05	0.9	0.05				5
Brain	2	0.45	0.8	0.05			30	10
Heart	4	0.25	2	0.05				90
Intestine	0.6						35	35
Kidney	5	0.3	5	0.08	25	2	60	20
Liver	9	$0.6 \pm 0.3^*$	6	0.06	20	7	170	110^*
Muscle	4	0.15	5.5	0.04			10	90
Ovary								90
Pancreas	6	0.25	3		20	13	115	45
Skeleton	0.3	0.8	10	12^*			150	30
Skin	1							100
Spleen	9	0.15	4.5	0.03	47	4	32	
Thyroid	6							$35,000^†$
Teeth	10	1.2	25	20^*				

¹ Blood values for several other trace minerals are given in the text.

² In women, 44 ± 2 mg. per cent.

³ About 70 per cent of the blood iodine is organic in nature.

⁴ In women, 0.10 ± 0.04 mg. per cent.

⁵ Cattle livers contain 3.6 ± 1.5 mg. per cent.

⁶ These values fluctuate considerably.

⁷ Equal to 35 ± 5 mg. per cent.

anion which can permeate cells. The ferric-globulin complex does not flocculate protein, like free ferric cations, nor does it have the narcotic effects of the ferrous cation. Intravenously injected ferric salts, ferricyanides, ferrocyanides, ferrigluconate, and ferricitrate do not function entirely like the ferric-globulin complex. The ferric salts permeate cell membranes poorly, but are partially stored in the liver and spleen; the extraneous complexes are excreted in the urine. Small parenteral doses of ferrilactate or ferrocitrate are partially oxidized and their iron is retained. After the ingestion of a soluble salt of radioactive iron (Fe^{59}), the plasma iron rises to a peak of 0.4 mg. per cent in from one to four hours, and returns to normal in from six to twelve hours. In anemic dogs, some of the administered radioactive iron is incorporated into the hemoglobin of circulating erythrocytes within four hours after ingestion. Erythrocytes show no exchange with radioactive iron *in vitro*. The serum iron rises when hemoglobin production is subnormal, and after slight hemorrhages; it falls during rapid formation of hemoglobin, in iron-deficiency anemias, and after severe hemorrhage. Normal human cerebrospinal fluid contains approximately 0.03 mg. per cent of iron.

Approximate concentrations of tissue iron are given in Table 102. Iron is present in cytoplasm and in lower concentration in chromatin, as porphyrin-complexes, protein complexes, and poorly dissociated ferric compounds of nucleoproteins and complex lipides. The iron concentration of the skin is lowered in squamous cell carcinomas. Cells of the kidney, liver, and spleen contain stored iron in the form of colloidal ferric hydroxide (hemosiderin). The ferric hydroxide combines with a protein (apoferritin) in the spleen to form ferritin. In normal animals, this protein-ferric hydroxide complex contains about 20 per cent of iron. Ferritin has been isolated from bone marrow, kidney, liver, and testicle. It is evident that the ferric iron of the plasma globulin complex is partially reduced in cells to form porphyrin-complexes; this reaction is especially prominent in erythroblasts. Of the 4.2 gm. iron in a 65 kg. adult human, approximately 57 per cent is present as hemoglobin; 20 per cent is storage iron; 16 per cent is parenchymatous iron (cytochrome, oxidases, etc.); and 7 per cent of the total, or one third of the muscle iron, is in myoglobin. Iron absorbed from the intestine, and the 27 mg. which are liberated daily in the normal adult by the catabolism of 8 gm. hemoglobin, are retained temporarily by the liver, spleen, and bone marrow in the form of ferritin. This stored iron is subsequently transported to the bone marrow and used for the synthesis of hemoglobin. The iron content of ferritin falls markedly after hemorrhage. Utilization of iron is inhibited in aplastic anemia, leukemia, and anemias resulting from chronic infections. Iron storage is very important, since the normal daily iron intake of the adult does not exceed 15 mg. The embryonic liver has a higher iron content than the liver of the adult. Female animals tend to store more iron than males; castration abolishes this difference. Myoglobin and the

parenchyma iron of the general tissues are not available for hemoglobin manufacture; their concentrations remain constant during anemia. When inorganic iron compounds are injected into tissues, they tend to remain at the site of injection for long periods, while injected hemoglobin is absorbed more readily. A fraction of the hemoglobin injected into tissues is rapidly converted to inorganic iron compounds *in situ*, and this iron is absorbed slowly. The iron present as hemoglobin in transfused erythrocytes can be used much more rapidly, and blood transfusion is regarded as the most efficient intravenous iron therapy.

Most mammals are born with appreciable iron stores; the guinea pig is a notable exception. The body of the newborn infant contains about 350 mg. of total iron. A considerable fraction is storage iron, inasmuch as the newborn has only 135 mg. of hemoglobin iron and 40 mg. of muscle iron. Studies with radioactive iron show that the plasma iron permeates the placenta readily; during early stages of pregnancy, approximately 0.4 mg. of iron is transferred daily from the mother to the embryo; in the last trimester the average transfer is 4.7 mg. per day. Since most of the iron deposition occurs during the last few months of fetal life, the premature infant has a deficient store. The iron supply of the normal infant is sufficient for one-half year. Such iron-containing foods as egg yolk, liver, meat, fruits, and vegetables must be added to the diet gradually; milk is notoriously poor in iron. Infants develop a negative iron balance readily, and they require iron therapy to raise their falling hemoglobin levels. About 0.5 mg. iron must be retained daily between infancy and maturity.

The functions of hemoglobin in the transport of carbon dioxide and oxygen have been considered on pages 21 and 543, the carrier and oxidase activities of tissue iron-porphyrin complexes on pages 97 to 100, and the chemistry of these iron complexes on pages 525 to 534.

The excretion of iron and other heavy metals is slow. Feces of fasting human adults contain about 8 mg. iron daily, and 15 mg. with ordinary diets. Approximately 0.2 mg. of iron per day is excreted in the urine. During an average menstrual period, 17 mg. of iron is lost as hemoglobin. Very little iron is secreted in milk.

Copper

This mineral is determined colorimetrically in ashed biological material by treatment with sodium diethyldithiocarbamate or with dithizone. The best dietary sources of copper are oysters, liver, molasses, mushrooms, nuts, and cereals (Table 101). The copper content of plant foods tends to vary with the concentration in the soil. The average daily copper intake of the adult is from 2 to 3 mg.; the suggested daily allowances are 0.03, 0.1, and 0.05 mg. per kg. of body weight for adults, children, and infants, respectively. Deficiency of the mineral interferes with growth, erythropoiesis, and hemoglobin formation in mammals. Rats reared

solely on milk, or milk plus highly purified iron salts, become severely anemic. This form of anemia is cured by including traces of copper salts in the diet; the quantities present in ordinary chemically pure iron salts are sufficient. Copper deficiency in cattle is termed *lecksucht* or falling disease, while in sheep and lambs it is known as coast disease or bush sick, and as enzootic ataxia or swayback, respectively. Swayback bears certain resemblances to Schilder's disease of humans. The symptoms of copper deficiency include diarrhea, emaciation, central nervous pathology, and anemia (which is hyperchromic and macrocytic in ewes and hypochromic in lambs).

Whole blood contains 0.12 ± 0.03 mg. per cent of copper, one half of which is present as hemocuprein in the erythrocytes. Milk has from 0.03 to 0.05 mg. per cent (Table 102, page 605). Administration of radioactive copper salt shows that the mineral is readily absorbed; the plasma copper level reaches a peak in from two to five hours, and subsequently copper accumulates in the erythrocytes. Traces of copper are found in all tissues, but the largest concentrations occur in the teeth, bony skeleton, and liver. The major portion of the body copper is found in bone, skin, and muscle; but the most active depot is the liver. The organs of the fetus and infant have greater copper concentrations than those of adults. The concentrations of copper are low in hepatomas, and in the kidneys of tumor-bearing animals.

Copper is not a component of the hemoglobin molecule, but it is present in erythrocytes and liver cells as the blue protein complexes, hemocuprein and hepatocuprein (page 535). Ascorbic acid oxidase and the phenol oxidases are similar copper-protein complexes, and the effects of copper on hemoglobin synthesis may represent the activity of some copper-containing enzyme. The bone marrow copper concentration does not increase during rapid erythropoiesis. Animals maintained on copper-free diets undergo depigmentation of fur. (See relation of copper to dopaoxidase and melanin formation, page 428.) Copper is necessary for the biological formation of catalase, peroxidases, cytochrome *a* and cytochrome oxidase; its presence in hemocyanins and turacin has been mentioned previously (pages 535 and 527).

Copper is excreted principally by the liver and intestine; biliary calculi at times contain as much as 300 mg. per cent. Only 0.25 mg. is excreted daily in the urine.

Cobalt and Nickel

Nickel is not at present regarded as an essential mineral, but it will be considered with cobalt because of similarities in the biological distribution and chemical properties of these minerals. A cobalt deficiency, Denmark disease, occurs in cattle and sheep. It is characterized by anemia, emaciation, and defective reproduction and lactation. Synonyms for similar

deficiencies in various domestic animals are: enzootic marasmus, bush sickness, salt sick, coast disease, pine disease, vinguish. Coast disease represents a dual deficiency of copper and cobalt. The essential biological function of cobalt is unknown. When excess cobalt salt is administered to dogs, rats, or rabbits, erythropoiesis is stimulated and marked polycythemia develops if the iron and copper supplies are adequate for the excessive hemoglobin production. In cobalt poisoning, tissue cysteine appears to be inactivated through complex formation with the metal, and administration of cysteine, cystine, or methionine is therefore beneficial.

Cobalt predominates over nickel in animal tissues (Table 102, page 605), while plant tissues show a reverse relationship. Buckwheat, figs, liver, and pancreas contain from 15 to 30 γ per cent cobalt; and buckwheat, figs, and peas contain from 100 to 200 γ per cent nickel. Other legumes, cereals, fruits, and nuts have smaller concentrations of both minerals. Cobalt and nickel are stored chiefly in the liver and spleen. Nickel is excreted in the feces and urine, and cobalt chiefly in the urine. Cobalt and nickel can replace manganese in the activation of arginase.

Manganese

This mineral is essential for the growth of animals and plants. The best dietary sources of manganese are blueberries, cereals, and nuts (Table 101, page 604). The concentrations in plant tissues vary with those in the soil. About 4 mg. manganese are ingested daily by the human adult. The daily requirement for children is estimated as 0.25 mg. per kg. of body weight. The slow intestinal absorption of this cation is hindered by excess calcium or phosphate in the diet, also by achlorhydria. Traces of manganese are found in all human tissues (Table 102, page 605). The 15 γ per cent in blood is present mainly in the erythrocytes. The blood level is not elevated appreciably during manganese poisoning; injected manganese salts leave the blood stream rapidly. The liver and bony skeleton are the most important storage depots, although some manganese is deposited in the muscle, skin, spleen, and pancreas. Tissues of birds have much greater concentrations of manganese than do mammalian tissues; the bodies of crawfish contain 10 mg. per cent.

Manganese is necessary for optimal growth and reproduction, but it is not required for the synthesis of hemoglobin. Deficit of the mineral causes stunted growth, diminution of hepatic arginase activity, defective coordination, leg deformity, disturbance of the estrus cycle, and testicular degeneration in rats and rabbits, and low hatchability, embryonic chondrodystrophia, micromelia, and perosis (bone deformities and slipped tendon) in chicks and turkeys. The blood and bone phosphatase activities are subnormal in perosis, and manganese activates certain tissue phosphatases (page 597). Manganese also acts as activator for arginase, dipeptidase, leucylpeptidase, oxaloacetic carboxylase, phosphoglucomutase,

and prolidase. About 85 per cent of the manganese excretion occurs in the bile and feces.

Zinc

Deficiency of zinc causes hyperirritability, retardation of growth, loss of hair, and decreased intestinal phosphatase activity in rats and mice. The adult human ingests from 12 to 20 mg. zinc daily; oysters are particularly rich in zinc. The mineral is present in drinking water supplies. The zinc content of a few foods is given in Table 101, page 604. An intake of 0.3 mg. zinc per kg. of body weight appears to be sufficient for young children; clinical deficiencies are unknown.

The plasma zinc concentration is 0.4 mg. per cent; the erythrocytes contain double this quantity. Zinc is present in the red cells as the enzymatic zinc-protein complex, carbonic anhydrase, which contains 0.33 per cent of the mineral (page 21). Another zinc complex is hemocytin, the blood respiratory pigment of the snail (sycotypus). The body of an adult man contains about 2.2 gm. zinc. Tissue concentrations of this mineral are given in Table 102, page 605. The nuclei of cells have a higher zinc concentration than the cytoplasm. The chief storage depots are the bony skeleton and liver; teeth and hair also contain relatively large concentrations (25 and 16 mg. per cent, respectively). It has been suggested that insulin is stored in the pancreas as a zinc complex. The diabetic pancreas contains only one half as much zinc as the normal organ. Zinc-insulin and zinc-gonadotropic hormone preparations are used to provide slow parenteral absorption of these hormones. The skin of beri-beri patients has a low zinc content. The mineral is excreted largely in the pancreatic and intestinal juices; normal human urine contains only 0.3 mg. of zinc per day.

Iodine

Total iodine can be determined in biological material by conversion to iodide through alkaline ashing, oxidation of the iodide to iodate, and titration of the latter with thiosulfate. Iodide is the only mineral trace anion which is recognized as a dietary essential of mammals. Deficient iodine intake leads to colloid enlargement of the thyroid; and the administration of iodide prevents and relieves colloid goiter (page 633). The minimal daily iodide requirement of the adult is approximately 25 γ and the optimal intake is about 150 to 300 γ per day. The iodine requirement is increased during growth, pregnancy, lactation, and infection. Dietary iodine is derived largely from sea foods, meats, certain fruits, vegetables, and drinking water (Table 101, page 604). Since the iodine content of foods and water varies considerably in different localities, the relative concentrations of the food iodine given in the table are more important than the specific values listed. Drinking water usually contains less than

0.2 γ per cent iodine; in goiter districts, where the iodine supply is inadequate, the concentration may be less than one tenth of this value. Iodized salt, marketed in the United States, contains about 20 mg. per cent of sodium or potassium iodide. Prophylactic administration of iodide is at times harmful to patients with adenomatous goiter. Iodides, iodized fatty acids, and the iodine containing amino acids, diiodotyrosine and thyroxine, are absorbed rapidly.

Iodide is transported by the blood and lymph. The blood iodine level rises rapidly after ingestion of iodide. The normal total iodine content of human blood is $8.5 \pm 3.5 \gamma$ per cent. About 95 per cent of the blood iodine is in the plasma. From two thirds to three fourths of the total blood iodine is organic in nature (diiodotyrosine, thyroxine, etc.); this fraction is insoluble in alcohol and about 85 per cent of the plasma iodine is bound to protein, especially to serum albumin. Immunological tests indicate that thyroglobulin does not appear in blood except after mechanical trauma to the thyroid gland. In hyperthyroidism, the blood iodine level may be as high as from 80 to 100 γ per cent. It is increased slightly during pregnancy, menstruation, and exercise, and during the summer. Blood iodine is as low as from 4 to 6 γ per cent in hypothyroidism. It is the organic iodine fraction which is chiefly affected by thyroid diseases, although some elevation of inorganic iodide occurs in hyperthyroidism. The iodine content of normal cerebrospinal fluid is 0.5 γ per cent. Tissue concentrations of total iodine are given in Table 102, page 605. From 90 to 110 γ per cent of total iodine are found in the liver, spleen, muscles, and ovaries; the thyroid normally contains much more (approximately 35 mg. per cent). It normally assimilates as much as 50 per cent of the dietary iodide from the blood stream. Metastatic thyroid carcinomas assimilate iodide very rapidly. Iodine normally constitutes from 0.3 to 0.4 per cent of the thyroid hormone, thyroglobulin, and 65 per cent of the active amino acid unit, thyroxine. About one half the body iodine is present in the muscles, and one fifth in the thyroid (0.1 mg. in the newborn and 10 mg. in the normal adult). The acid insoluble thyroxine iodine constitutes 40 ± 10 per cent of the total iodine in the normal thyroid. Diiodotyrosine and inorganic iodide, which are acid soluble, form 53 ± 10 , and 7 per cent, respectively, of the thyroid iodine. During hyperplasia of the thyroid, the total thyroid iodine and thyroxine decrease markedly; involution of the gland with formation of a colloid goiter tends to restore normal concentrations.

Treatment of rats with thyrotropic hormone of the anterior pituitary gland doubles the speed of conversion of administered radioactive iodide to organic iodine compounds. Hypophysectomy has an opposite effect, causing the formation of less thyroxine in the thyroid gland and a lowered plasma level of thyroxine-like compounds. Conversion of iodide to diiodotyrosine is affected less by hypophysectomy. The synthesis of iodo amino acids in the thyroid gland is inhibited by azide, *p*-aminoaromatic

acids, carbon monoxide, cyanide, sulfide, sulfonamides, thiouracil, and thiourea. Thiocyanate exerts a similar effect, but it can be reversed by iodide. Azide and sulfonamides do not interfere with assimilation of iodide by the gland, while carbon monoxide, cyanide, sulfide, and thiouracil inhibit both assimilation and synthesis of thyroxine in thyroid slices. The administration of thyroxine prevents the effects of sulfonamides and thiouracil on the gland. Studies with radioactive iodide in thyroidectomized animals have revealed the synthesis of small quantities of diiodotyrosine and thyroxine by such tissues as liver and intestine.

About 0.3 mg. of thyroxine is catabolized daily in the human adult. The 0.2 mg. iodide, which is thereby formed, is retained largely in the body and is utilized for resynthesis of thyroglobulin. Administered diiodotyrosine is converted rapidly to inorganic iodide. The iodide anion diffuses rapidly into body fluids; soon after its ingestion, iodide can be found in milk, gastro-intestinal secretions, saliva, sweat, and tears. Injected radioactive iodide is distributed rapidly throughout the extracellular fluid; it appears in the gastric juice as hydriodic acid. There is an enterohepatic circulation of iodide; after iodide administration, the biliary iodine is elevated above the fasting level ($9 \pm 5 \gamma$ per cent), and the excreted mineral is reabsorbed in the small intestine. About 75 per cent of ingested iodide appears in the urine within 12 hours. Approximately 50 γ of iodine are excreted daily in the urine of a normal adult. Day urine contains more iodine than night urine. Only small quantities of iodine are found in the feces. In hyperthyroidism, the urinary and fecal iodide output is increased, unless excessive perspiration causes oliguria. The urinary iodine excretion is increased slightly in pregnancy, during menstruation, and following partial thyroidectomy.

NON-ESSENTIAL TRACE ELEMENTS

The storage depots and excretory organs for the trace minerals which are not considered in detail in this section are given on pages 569 and 571.

Aluminum

Traces of aluminum are found in plants and in animals. The average concentration in normal human blood is 13 γ per cent. Animal tissues contain less than 0.2 mg. per cent; the largest quantities occur in the skin, lungs, and intestine. Since aluminum salts are absorbed very poorly, their ingestion usually does not cause any toxic effect. A sufficiently large dose interferes with phosphate absorption and causes rachitis.

Bismuth

Bismuth salts are absorbed with great difficulty. When injected they accumulate principally in the kidneys, liver, large intestine, and bone. They are excreted largely in the urine.

Lead

Small quantities of this mineral are present in foods. The concentrations in plant foods tend to vary with the content of lead in the soil; they can be increased considerably by insecticide treatment. Small quantities of lead are also introduced into meat, fish, and plant foods during industrial processing. Modern city water supplies contain some lead, derived from pipes (at times as much as 0.25 mg. per cent, usually much less). The average intake of humans is 0.3 mg. per day.

Normal blood contains about 0.03 ± 0.02 mg. per cent lead, largely in the erythrocytes. Most animal tissues have less than 0.1 mg. per cent; normal bones contain as much as 2 mg. per cent. Factors which influence the storage and mobilization of calcium have similar effects on lead metabolism. The chief lead storage depot is the bony skeleton; the liver also stores small quantities. Lead is excreted both by the intestine and the kidneys. The excretion of lead is accelerated by acidifying measures and by low calcium intake. Lead poisoning leads to anemia and accumulation of coproporphyrin in the bone marrow, feces, and urine (page 563).

Molybdenum

Peas and beans are reported to contain more of this mineral than other plant foods, namely, from 0.6 to 0.9 mg. per cent. Only traces of molybdenum are found in mammalian tissues; the largest concentrations (less than 0.1 mg. per cent) are present in the liver and spleen. Molybdenum is excreted largely in the feces.

Thallium

This mineral is deposited chiefly in the liver, spleen, and kidney. It is excreted largely in the urine. Thallium poisoning causes decalcification of bone.

Tin

The tin content of most animal tissues is variable. The mineral has been reported in especially large concentrations in the tongue (from 1 to 2.5 mg. per cent) and skin (from 0.5 to 1 mg. per cent). Normal blood contains 12 γ per cent, chiefly in the erythrocytes; the liver and brain have less than 0.25 mg. per cent. Tin salts are excreted largely in the feces.

Arsenate

Most foods contain only small traces of this mineral; sea foods have somewhat larger quantities. About 0.1 mg. per cent of arsenic is found in human tissues. The liver and kidney are the chief arsenic depots; but arsenates can accumulate in the hair, nails, skin, and thyroid. Both arsenate and antimonate are retained within the body for long periods

before they are excreted by the intestine and kidneys (small amounts are also excreted by the skin). Arsenic poisoning produces gastro-intestinal and hepatic symptoms. Tolerance to arsenic can be established by gradually increasing the dose. Arsenite inhibits certain dehydrogenases.

Bromide

Ordinary table salt contains about 165 mg. per cent bromide, and edible plant tissues from 0.1 to 2 mg. per cent. Since bromide is readily absorbed in the intestine and distributed rapidly throughout the body fluids, the distribution of this anion can be used to estimate the volume of extracellular fluid. Normal human serum contains 1 ± 0.7 mg. per cent of bromide, and the erythrocytes have one half this quantity. The bromide concentration in extracellular fluid is of the same order as the plasma concentration. Administered bromide tends to replace body chloride. When sufficient quantities of bromide are ingested, the anion exerts a narcotic effect which is not produced by chloride deficit. The thyroid gland exhibits a selective affinity for both radioactive bromide and radioactive iodide. Bromide is excreted largely in the urine. (See bromism, page 634.)

Fluoride

This mineral occurs in all foods and in many mineral waters. Cereals, fruits and vegetables contain from 0.02 to 2.2 mg. per cent; the quantity varies with that in the soil. Fluoride is absorbed readily and is transported in the blood, which normally contains 0.05 ± 0.03 mg. per cent. Bones and tooth enamel contain much more fluoride than soft tissues (about 12 and 20 mg. per cent, respectively). The bony skeleton is the chief storehouse of fluoride, although in chronic poisoning (fluorosis) the anion also accumulates in the soft tissues (page 635). Fluoride inhibits the activity of phosphatases; the mineral is excreted principally in the urine.

Silicate

This mineral can be determined colorimetrically, in ashed biological material, as silicomolybdic acid. Food silicates are derived chiefly from vegetables. Herbivorous animals ingest from ten to thirty times as much silicate as carnivora. The human adult ingests several hundred milligrams of silica daily. Soluble silicates are absorbed readily in the intestine. Human blood normally contains about 1.5 ± 1 mg. per cent (as silicon dioxide); this level is not elevated readily by ingesting silicate or by inhaling silica dust, even though the urinary excretion is increased. After injecting silicate, the blood level remains high for weeks. The silicate content of the human body varies considerably. The mineral is found in all tissues, particularly in the lung, kidney, pancreas and skin. In adults, the lungs contain the largest quantities; the silicon content can be in-

creased tremendously by exposure to silica dusts (silicosis). Silicosis is characterized by a specific tissue reaction, which leads to fibrotic lesions and local accumulation of phospholipide. Skin silica decreases with age. Silicates are excreted in the urine and in the feces.

Titanium

Traces of titanate are distributed widely in plant and animal tissues; especially large quantities occur in certain crustacea. Concentrations of from 1.5 to 11 γ per cent have been reported in mammalian tissues; the highest levels are found in the bones, lungs, hair, and liver. Titanates are excreted in the urine.

PATHOLOGY

*"What am I, Life? A thing of watery salt
Held in cohesion by unresting cells
Which work they know not why."*

JOHN MASEFIELD

ABNORMAL BLOOD VOLUME

Decreased blood volume (hypovolemia) is usually accompanied by transudation of water and electrolytes and abnormal concentrations of plasma proteins. In surgical shock resulting from hemorrhage or trauma, the blood pressure and blood volume are greatly lowered, the circulation time is prolonged markedly, the abdominal vessels are dilated, and the capillary permeability is increased, with subsequent transudation of fluid from the blood. The serious hemoconcentration which results can be detected by high hematocrit values and elevated erythrocyte counts. After severe hemorrhage, the plasma volume is restored more rapidly than the blood volume. Hepatectomy reveals a considerable increase in protein catabolism and carbohydrate utilization by peripheral tissues in experimental hemorrhagic shock. It has been claimed that one of the factors in the production of traumatic shock is the liberation of adenosine triphosphate from crushed tissues. This substance is not concerned in shock caused by circulatory occlusion. Hemoconcentration and low blood volume also result from prolonged water restriction, diarrhea, external fistulae, profuse sweating, diuresis, metabolic acidosis, adrenal cortical insufficiency, and myxedema. Adrenalectomy and thiamin deficiency increase the susceptibility to shock. Since considerable plasma protein leaves the blood during severe shock, the condition can be improved markedly by intravenous administration of plasma, plasma protein concentrate, or serum albumin, and by transfusion in the case of hemorrhagic shock. Injection of gum acacia increases the oncotic pressure and blood volume, but this treatment has certain undesirable features (page 444).

Administration of corticosterone is useful at times in the treatment of surgical shock. In pernicious anemia (but not in most other chronic anemias), the blood volume decreases, while in polycythemia vera it increases markedly (hypervolemia). The blood volume can rise to twice the normal value in severe congestive heart failure. It is increased in leukemia, hepatic cirrhosis, Banti's disease, and hyperthyroidism. A 20 per cent increase occurs during normal pregnancy, due largely to increased plasma volume. The blood volume is increased temporarily by excitement, muscular exercise, and high environmental temperature, owing to contraction of the spleen.

DEHYDRATION

Decrease in the volume of extracellular fluid is usually caused by severe water restriction, or by excessive loss of fluid, as in vomiting, diarrhea, polyuria, external fistulae, hemorrhage, burns, surgery, diabetes mellitus, fever, severe allergy, and infections of the upper respiratory tract. Dehydration is accompanied by rapid loss of body weight, tissue destruction, acidosis, renal hypofunction, fever, dryness of the skin, decreased plasma volume, lowered intraocular pressure, and increased blood viscosity, plasma protein concentration, non-protein nitrogen, erythrocyte count, hemoglobin content, and hematocrit reading. Coexisting anemia and protein deficiency mask the elevation of hemoglobin and plasma proteins. Severe dehydration, accompanied by partial loss of intracellular water, can rapidly be fatal, especially in infants. Since renal concentration and the urea and mineral clearances are subnormal in the newborn, the plasma composition is readily affected by diarrhea, vomiting, or fluid injection. The association of dehydration with acidosis has been discussed on page 39. Injection of saline, sodium lactate, and glucose solutions constitutes the chief therapy of dehydration. When ketosis is present, insulin may also be required. Plasma injection is indicated in severe burns, and transfusion of whole blood in hemorrhage.

The ingestion of water after excessive sweating induces the condition known as heat cramps or miner's cramps. A sodium chloride deficiency is produced by excessive perspiration, and the urine becomes almost chloride-free. The administration of sodium chloride relieves this condition and the similar syndrome which arises in pyloric obstruction from excessive vomiting. Anhydremia, or decreased concentration of plasma water, occurs only after the other extracellular fluids are largely exhausted. Marked diuresis and a form of dehydration known as salt fever are produced by the administration of hypertonic salt or sugar solutions; the body temperature increases markedly, and the spinal fluid pressure is lowered. Dehydration by intravenous hypertonic sucrose, glucose or saline solution is sometimes used in brain surgery to decrease cerebral edema and lower intracranial pressure. Water loss during starvation differs from ordinary dehydration;

under these circumstances, two thirds, or more, of the excreted water is derived from the cells.

EDEMA

In this condition there is an accumulation of extracellular tissue fluid, the blood volume and intracellular water remaining relatively normal. Edematous swelling and fluid accumulation can be either local or generalized; it occurs chiefly in the soft connective tissue and body cavities. Compact or rigid structures imbibe relatively little fluid. The swelling of such protein fibers as collagen, elastoidin, hair, myosin, and silk is minimal over a wide pH range (from 5 to 7 for muscle, from 4.5 to 8 for collagen, and more extensive ranges for the other fibers mentioned). The extracellular fluid can be more than doubled in edema. Under pathological circumstances, the pleural cavity may contain more than 2 liters of water, and the abdominal cavity more than 5 liters. Accumulation of serous fluid in the peritoneal cavity is termed ascites; the most frequent causes of this condition are hepatic cirrhosis, cardiac and renal disease, peritonitis, abdominal tumors, and obstruction of the portal circulation or inferior vena cava. Edema of the brain is associated with eclampsia, uremia, acute alcoholism, and cerebral trauma. It can be produced by massive intravenous injections of hypotonic salt solution. Pulmonary edema is incited by toxic substances, and by processes which cause shock.

Important factors in the production of edema are: (a) lowered oncotic pressure of the plasma, caused by loss of plasma albumin (nephrosis, nephritis, massive hemorrhage, and plasmapheresis); by inadequate protein intake (malnutrition and gastro-intestinal diseases); by impaired synthesis of plasma protein (anemias, chronic infections, hepatic disease, nephritis and other cachexial conditions); (b) increased capillary blood pressure resulting from congestive heart failure, venous obstruction, and vasodilatation; (c) transudation of plasma protein due to increased capillary permeability (acute glomerulonephritis, angioneurotic edema, anoxia from anemia or congestive heart failure, burns, infections, inflammation, pulmonary edema, and urticaria); and (d) decreased lymphatic drainage in elephantiasis, liver disease, and so forth. The edema fluid which results from decreased oncotic pressure has a low protein content, while the fluid caused by increased capillary permeability usually contains more than 1 per cent protein (Table 12, page 62). High intake of sodium and water is an important contributory factor, one which accentuates most types of edema. A normal person can accumulate some edema fluid by ingesting from 35 to 40 gm. sodium chloride daily. Restriction of salt intake is frequently used to control clinical edema, pre-eclamptic conditions and tuberculosis of the skin. Water, sodium and chloride are usually retained, or eliminated, together. In pneumonia, sodium chloride is

stored with little tendency toward general edema; the retained salt is excreted rapidly following the crisis.

The fluid transfer between blood and tissues is normally in a state of equilibrium; water leaves the plasma at the arteriolar side of the capillaries under the driving force of the hydrostatic blood pressure (approximately 32 mm. mercury at the arteriolar end of the capillaries, and 12 mm. at the venous end). Fluid from the tissues enters the blood at the venous end of the capillary, owing to the oncotic pressure of the plasma proteins (28 ± 2 mm. mercury). The hydrostatic and oncotic pressures of tissue fluid are equal to 8 and 10 mm. mercury, respectively. Adult nephritic patients develop palpable edema when the plasma specific gravity is less than 1.023, the plasma protein level is below 5.0 ± 0.5 per cent (oncotic pressure less than 18 mm. mercury), or the plasma albumin level is below 2.5 ± 0.5 per cent. In nephrotic children, the critical albumin level is about one half this value. Each per cent of plasma albumin exerts an oncotic pressure of 7.54 cm. water or 5.54 mm. mercury, as compared with 1.95 cm. water or 1.43 mm. mercury for each per cent of globulin. Each gram of injected serum albumin can attract 17.5 ml. of edema fluid into the blood stream, but it does not diminish ascites. Nephrotic patients excrete considerable fractions of injected serum albumin in the urine.

The term "nutritional edema" (war, famine, or hunger edema) is applied to edema caused by hypoproteinemia from inadequate protein and caloric intake. It occurs, at times, in cardiac, diabetic, and nephritic patients on restricted diets, and in chronic alcoholism, anemias, intestinal disease, pellagra, pregnancy toxemias, scurvy, and tuberculosis. Low protein intake is an important general factor in edema production. During severe ketosis in diabetic patients, the plasma protein depletion caused by restricted diets can be masked by hemoconcentration. When the normal plasma volume of the patient is restored by active treatment with insulin, glucose, and saline, marked edema may develop from hypoproteinemia. As much as 4 liters of water can accumulate in the body before edema is detected by physical examination. Development and subsidence of edema are investigated more accurately by comparing the daily fluid or beverage intake with the urinary output. In normal persons, these volumes usually balance within 5 per cent. The edema of thiamin deficiency is dependent in type, but it is not due to cardiac failure or hypoproteinemia.

That failure of water elimination by the kidneys is not a fundamental factor in edema has been proved by nephrectomy. In nephritis and nephrosis, there is a tendency toward prerenal diversion of water to the extracellular fluids. Nephritic edema is usually the result of lowered plasma protein, but it can be induced by myocardial weakness or capillary injury. In terminal stages of glomerulonephritis, edema is frequently superseded by anhydremia due to vomiting, diarrhea, and acidosis; under these circumstances, the plasma protein depletion is masked, and the

plasma protein concentration is nearly normal. Oliguria in nephritis and nephrosis is often due to edema, vomiting or fever, while polyuria generally represents a compensatory mechanism to impaired renal concentrating ability. In myxedema, water retention is accompanied by a peculiar extracellular accumulation of protein. Following the administration of thyroïd, this protein is catabolized, and the excess extracellular water and sodium chloride are excreted.

The ingestion of alkali usually diminishes water excretion, while the administration of inorganic and metabolically inert organic acids causes diuresis. Physiological sodium chloride solution can act as a mild diuretic in normal persons, and intravenous hypertonic saline or sugar solutions act as powerful diuretics which temporarily remove edema fluid. Potassium salts ordinarily act as diuretics, since they are not easily stored in the extracellular fluid. When they fail to be excreted, potassium salts aggravate edema and cause toxic symptoms (depression, cyanosis, lowered blood pressure), especially in patients with cardiac decompensation, nephrosis or nephritis. Purine, mercurial, and acidifying diuretics fail to exert their ordinary diuretic action in severe renal insufficiency. In general, the use of diuretics and laxatives, and the aspiration of fluids, are purely secondary measures for combating edema; successful treatment requires correction of the underlying pathology. The administration of digitalis in cardiac edema, of protein and thiamin in nutritional edemas, of thyroïd in myxedema, and the elimination of sensitizing agents in the urticarias are fundamental therapeutic measures. Dairy proteins are substituted for meat in diets for patients with ascites (page 560). Edema is usually increased by erect posture; physical activity diminishes edema only when cardiac disease is absent and lymphatic drainage is satisfactory. Cardiac and nephritic edemas are increased by exercise.

POLYURIA; DIABETES INSIPIDUS

Marked polyuria can occur in diabetes insipidus, epilepsy, hysteria, migraine, paroxysmal tachycardia, pyelitis, cerebral crises of hypertensive disease, and diabetes mellitus. The urine of diabetes mellitus patients has a high specific gravity and contains sugar. As mentioned on page 450, there is a relative polyuria in nephritis, because of deficient concentrating ability of the kidneys.

Lesions in the vicinity of the hypophysis lead to degeneration of the supra-optico-hypophysial tract and the pars nervosa of the pituitary, and cause the development of the condition known as diabetes insipidus. In this condition, the posterior portion of the pituitary becomes deficient in pitressin. The deficiency allows unbalanced metabolic activity of the anterior lobe hormones and pronounced polyuria. Excision of the entire pituitary does not produce diabetes insipidus. Pitressin is necessary for normal reabsorption of water and concentration of urine by the renal

tubules. In severe diabetes insipidus, as much water can be excreted daily as is normally present in the entire body (40 liters). The cardinal symptoms of diabetes insipidus include polyuria, polydipsia, nocturia, and cachexia. The urine has a very low specific gravity; glycosuria does not occur, and the blood chemistry is essentially normal. The condition does not incite renal pathology or fatigue of the kidney, even after many years of continued diuresis. Posterior pituitary extract (pitressin) relieves all the symptoms temporarily, but does not cure diabetes insipidus. The hormone is, therefore, given daily as replacement therapy. Application of a local anesthetic to the mucous membrane does not abolish the abnormal water intake and polyuria. Limitation of the water intake causes great discomfort and serious uremic symptoms, since only dilute urine can be secreted by the patients. The administration of sodium chloride aggravates the polyuria, and limitation of salt decreases it (page 680).

WATER INTOXICATION

This condition is of minor clinical interest. It has been produced in dogs by the administration of sufficient water to exceed the renal excretory ability. The symptoms of water intoxication include weakness, nausea, incoordination, polyuria, diarrhea, vomiting, salivation, convulsions, and coma. The blood pressure and intracranial pressure increase, and the serum chloride, potassium and sodium concentrations fall. There is relatively little edema or hypervolemia. Intravenous injection of hypertonic saline solution relieves the symptoms.

HYPERCHLOREMIA

The chloride concentration of whole blood is of little clinical significance, since it is affected by the erythrocyte volume; in anemia, the chloride level of whole blood is inversely proportional to the red cell count. (See chloride distribution, Table 100, page 582.) Hyperchloremia, or high plasma chloride concentration, results from excessive hyperventilation, massive injection of saline in patients exhibiting marked oliguria, and occasionally from uremia and anuria.

HYPOCHLOREMIA

Decrease in plasma chloride frequently accompanies excessive vomiting (as in pyloric or upper intestinal obstruction, gastritis, uremia, and pregnancy toxemias). When vomiting is complicated by achlorhydria or achylia, there is less effect on the plasma chloride. The dehydration which accompanies vomiting and diarrhea frequently conceals the degree of chloride depletion; hence, hypochloremia is by no means a quantitative index to the chloride requirement of the patient. Chloride loss in uremia is, at times, aggravated by inability of the kidney to conserve the anion. Hypochloremia also occurs following repeated withdrawal of ascitic

fluid, and in hepatic cirrhosis, acute mercury poisoning, Addison's disease, heat cramps, meningitis, rheumatic fever, the precritical stage of lobar pneumonia, and, occasionally, in diabetes mellitus. Following the crisis of pneumonia, there is a marked increase in urinary chloride excretion, and the plasma chloride level is restored to normal. The hypochloremia of adrenal cortical insufficiency is accompanied by low plasma sodium, hypovolemia, circulatory insufficiency, and renal functional impairment. Continued administration of large doses of desoxycorticosterone results in adrenal atrophy and marked hypochloremia.

The *cerebrospinal fluid chloride* level fluctuates with that of the plasma. The plasma level must, therefore, be considered in the interpretation of spinal fluid chloride values. In most forms of meningitis, the level is low, especially in tuberculous meningitis. The low chloride concentration found in inflammatory conditions of the meninges is probably due to increased protein content of the spinal fluid.

PATHOLOGICAL SERUM SODIUM LEVELS

The serum sodium concentration is low in adrenal cortical insufficiency; at times after prolonged sweating, vomiting, or diarrhea; and in acidosis, diabetes mellitus, heat cramps, uremia and in pneumonia prior to the crisis. *Heat cramps* (stoker's or miner's cramps) is caused by excessive ingestion of water after profuse sweating. The symptoms resemble those of adrenal cortical insufficiency, namely, headache, muscular cramps, exhaustion, renal functional impairment, hemoconcentration, hypochloremia, and low plasma sodium. When sodium depletion is masked by dehydration, there is little or no lowering of the plasma sodium level.

Increased serum sodium levels are relatively infrequent; they have been reported in Cushing's syndrome (pituitary basophilism) in association with elevated plasma chloride and decreased plasma potassium concentrations. These changes, which are opposite to those encountered in Addison's disease, have been attributed to adrenocortical stimulation by excessive liberation of corticotropic hormone from the anterior hypophysis.

PATHOLOGICAL SERUM POTASSIUM LEVELS

Serum potassium is elevated in Addison's disease, and the cation accumulates in the erythrocytes and muscles. A high potassium intake aggravates the symptoms of adrenal insufficiency, whereas a low intake is beneficial. Serum potassium is also increased in asphyxia, acute intestinal obstruction, uremia, trauma, and acute hemolytic conditions (for example, the onset of a malarial chill). Small increases in serum potassium have been reported in acute allergic manifestations, and, at times, in cholera, typhoid, and pneumonia. Administration of potassium chloride occasionally affords temporary relief from allergic symptoms.

Serum potassium has been reported to be low in Cushing's syndrome. It is markedly decreased at the onset of seizures in familial periodic paralysis, a syndrome which is more common in males than in females. These attacks of bilateral flaccid paralysis of the extremities, which develop when serum potassium is less than 12 mg. per cent, last for hours, or even days. In some cases, more permanent weakness, bradycardia, and muscle dystrophy occur. Measures which lower the plasma potassium concentration (carbohydrate meals, insulin, or strenuous exercise) tend to induce attacks. During the paralytic attacks, the urinary potassium excretion declines and creatinuria can occur. The administration of potassium salts relieves the paralytic symptoms. The administered potassium is excreted quite rapidly. Excessive doses of desoxycorticosterone acetate produce in dogs a type of flaccid paralysis which resembles familial periodic paralysis.

ADRENAL CORTICAL INSUFFICIENCY (ADDISON'S DISEASE)

Acute cortical insufficiency, and periods of crisis in Addison's disease, are characterized by muscular weakness, fatigue, hypoglycemia, lowered oxygen consumption, decreased plasma sodium and chloride, hypovolemia, decreased blood flow, and increased erythrocyte count, hematocrit value, and plasma potassium, protein, non-protein nitrogen, phosphate, and sulfate concentrations. The terminal stage is characterized by circulatory collapse, fall in blood pressure, and lowered body temperature. The muscles contain increased quantities of intracellular fluid and potassium. There is an increased urinary elimination of sodium and chloride, and a relative retention of potassium. Crisis can be precipitated by the administration of diets low in sodium and high in potassium salts. High carbohydrate, low potassium diets, and the administration of sodium bicarbonate, chloride or citrate, and of cortical extract or desoxycorticosterone assist recovery. The patients continue to exhibit hypoglycemia and weakness unless whole cortical extract is used. Desoxycorticosterone, dehydrocorticosterone, and corticosterone allow normal tubular concentration of potassium and retention of sodium, while corticosterone, dehydrocorticosterone, and compound E improve the carbohydrate metabolism (page 702). Overdosage with desoxycorticosterone can cause such symptoms as cardiac insufficiency, edema, hypertension, and peripheral motor paralysis.

HYPERCALCEMIA

The serum calcium level is high in hyperparathyroidism (from 12 to 24 mg. per cent), also in hypervitaminosis D, polycythemia vera, extensive neoplastic disease of bone, multiple myeloma (due to hyperproteinemia), and uremia complicated by hyperparathyroidism. Slight hypercalcemia occurs in asphyxial conditions due to increased carbon dioxide tension.

The calcium content of cerebrospinal fluid increases only slightly in hyperparathyroidism, but the non-diffusible protein-calcium fraction is raised whenever the spinal fluid protein content is increased, as in meningitis, epidemic encephalitis, and brain lesions.

HYPOCALCEMIA

Hypoparathyroidism is the chief cause of clinical hypocalcemia; it causes a decrease in all serum calcium fractions. Removal of the parathyroids reduces the serum calcium to approximately one half its normal level, and it causes tetany and death. Serum calcium is low in tetany, whether the condition is primarily due to hypoparathyroidism, or is associated with rachitis or osteomalacia, dietary calcium deficiency, or with thyroidectomy (disturbance of blood supply to the parathyroids). Hypocalcemia can occur in the type of hyperparathyroidism known as renal rickets. In most cases of rachitis, the serum calcium is normal. Steatorrhea, celiac disease, and sprue interfere with the intestinal absorption of calcium, and they frequently cause hypocalcemia. The hypoproteinemia which accompanies nephrosis, chronic glomerulonephritis, and kala-azar causes hypocalcemia. In these conditions, the calcium diminution is almost entirely in the protein-bound calcium, and tetany does not occur. In late glomerulonephritis the serum calcium level often falls as the result of hyperphosphatemia. There is a decrease of approximately 1 mg. per cent in the serum calcium level during late pregnancy and labor. Hypocalcemia is of fundamental importance in the etiology of milk fever (puerperal paresis) of lactating cattle and sheep; this form of tetany is remedied by injection of calcium salt, or by inflation of the udder to inhibit secretion of milk.

HYPOPARATHYROIDISM (TETANY)

When the serum calcium level falls below 7 mg. per cent, and the calcium ion concentration is less than 3.5 mg. per cent, a condition of neuromuscular hyperirritability, known as tetany, usually appears. Tetany is manifested by convulsions, carpopedal spasm, and laryngospasm. Latent tetany, which is present at serum calcium levels of from 7 to 8 mg. per cent, can be detected by the Chvostek sign (reflex contraction on stimulation of the facial nerve) and the Trousseau sign (carpal spasm on constriction of the blood vessels of the arm). Blood analysis differentiates tetany from hyperinsulinism.

The rapidity of fall in the serum calcium ion concentration is an important factor in determining the onset of tetany. The calcium concentration of the brain is normal in tetany, and the cerebrospinal fluid calcium is decreased only 1 mg. per cent. Urinary calcium excretion is decreased when the serum calcium level is below 8.5 mg. per cent. (Sulkowitch's

buffered oxalate reagent is convenient for estimating the calcium excretion.) The lowered serum calcium may contribute to the motor irritative symptoms of uremia.

The syndrome of tetany appears, at times, in alkalosis, owing to diminution of the ionized serum calcium fraction, even though the total serum calcium is not subnormal. Tetany can result from the administration of excessive quantities of alkali in the therapy of peptic ulcer (especially if kidney function is subnormal), also from hyperventilation and excessive vomiting of acid gastric juice. In these conditions, the plasma bicarbonate increases and the ionized calcium is lowered. The injection of oxalate, or of large quantities of citrate or phosphate, produces similar decreases in calcium ion concentration. The total serum calcium concentration is high after the injection of citrate, and low after oxalate or phosphate injection.

Ordinary low calcium tetany can be prevented by the injection of curarine; but this substance does not abolish the low magnesium form of tetany, in which the serum calcium and calcium ion concentrations are normal. When serum calcium and magnesium are both lowered, tetany does not result. Fever, anoxia, and injection of phosphate aggravate tetany. The administration of calcium salts abolishes the symptoms of tetany, raises the serum calcium level, and increases phosphate excretion (chiefly in the feces). It is, therefore, important to provide diets with a high calcium : phosphate ratio in hypoparathyroid conditions. Aluminum hydroxide may be given to precipitate phosphate in the intestinal tract, and thereby minimize its absorption. To provide a low phosphate diet and decrease the fecal calcium loss, meat, dairy products, and nuts are kept at a minimum. Milk intake is reduced in infantile tetany; even though the milk phosphate is balanced by calcium, the latter is not well utilized. Ingestion of lactose, acids and acidifying diuretics affords relief by indirectly raising the serum calcium level. Vitamin D, thyroid and dihydrotachysterol (A.T. 10) are even more effective in increasing the serum calcium. Parathormone injection is very efficient; but, because of the formation of an antihormone, its therapeutic value diminishes on continued administration. Parathormone does not increase the calcium content of the brain (page 589).

HYPERPARATHYROIDISM

Symptoms of acute experimental hyperparathyroidism include lethargy, weakness, vomiting, and diarrhea. With the exception of the vomiting center, the central nervous system tends to be depressed by the marked hypercalcemia. The serum phosphate is first lowered slightly, as this anion is excreted in the urine, and the serum calcium and magnesium concentrations are increased. Later, the urinary excretion of calcium and phosphate is increased; fecal elimination is decreased. The serum phosphatase activity is increased. Calcium and phosphate are withdrawn from

the bony skeleton. Excessive doses of the hormone cause bone rarefaction and osteitis fibrosa cystica. The most rapidly growing bones are affected first; young animals are especially susceptible to the hormone. The calcium withdrawn from the bones is partly transferred to the soft tissues, where metastatic calcification occurs. Parathyroid extract exerts a diuretic action which leads to dehydration and hemoconcentration. The serum inorganic phosphate and the non-protein nitrogen of blood rise progressively, owing to functional impairment of the kidneys. High phosphate diets and alkali tend to ameliorate the condition, while high calcium diets and acidifying measures aggravate it.

Clinical hyperparathyroidism, associated with parathyroid tumor, is characterized by similar symptoms, namely, pain in the back and extremities, weakness, fractures, bone swelling and resorption, and renal symptoms. The bone abnormalities are relatively late manifestations of the disease. X-ray examination reveals decalcification of the bony skeleton, and the presence of cystic areas. The hypercalcemia, hypophosphatemia, calcinuria, and phosphaturia are readily masked or modified by renal impairment, and by dietary influences. In about three fourths of the patients the serum calcium concentration is above 12 mg. per cent. The serum phosphatase activity is increased in almost all cases. Surgery is the most reliable therapeutic measure, but it is frequently followed by tetany, even with serum calcium levels above 6 or 7 mg. per cent. Secondary forms of hyperparathyroidism accompany carcinomatous metastases to bone, multiple myeloma, chronic nephritis, osteomalacia, Paget's disease, Cushing's syndrome, rachitis, and renal rickets. Hyperparathyroidism leads to formation of urinary calculi and calcification of the renal tubules, associated with excessive urinary calcium excretion. (Endocrine relations in hyperparathyroidism are considered on page 711.)

Renal Rickets

This disease usually begins in childhood with symptoms of stunted growth, bone deformities, weakness, anemia, degeneration of the adrenal cortex, yellow pigmentation of the skin, polyuria, and polydipsia. There is marked retention of non-protein nitrogen and phosphate, decreased absorption of calcium, increased serum phosphatase activity, acidosis, hyperparathyroidism, and, at times, tetany, even though the blood calcium is near the normal level. Loss of calcium from the bony skeleton can result in marked deformity, dwarfism, and osteitis fibrosa. Renal rickets and parathyroid hyperplasia are associated with a wide variety of destructive renal lesions (nephritis, nephrosis, hydronephrosis, congenital polycystic kidney, cystinuria, etc.). Similar parathyroid hyperplasia follows experimental renal lesions in rats, and injection of phosphate in rabbits. Disuse of a limb can result in sufficient osteoporosis to simulate hyperparathyroidism and produce renal damage.

HYPERPHOSPHATEMIA

The hypocalcemia which accompanies advanced renal failure, tetany, and the injection of phosphate is accompanied by hyperphosphatemia. Retention of phosphate in renal insufficiency is of similar significance to increased blood creatinine; levels above 8 mg. per cent indicate a serious prognosis in chronic nephritis. In renal rickets, and in well developed hyperparathyroidism, the serum phosphate increases as kidney function becomes impaired. The hyperphosphatemia of tetany is accompanied by decreased urinary phosphate excretion. A slight hyperphosphatemia accompanies the healing of fractures in adults. The serum phosphate level of children undergoing rapid skeletal development is higher than that of adults (Table 100, page 582). The diphosphoglycerate of the erythrocytes increases in renal impairment and pyloric obstruction. In terminal nephritis, the adenosine triphosphate fraction of the erythrocytic phosphorus is elevated. Increases in organic acid-soluble phosphorus have been reported in leukemia, pernicious anemia, and in erythroblastosis neonatorum and severe jaundice of the newborn. The cerebrospinal fluid phosphate concentration increases in uremia, meningitis, and degenerative processes of the brain and cord. *

HYPOPHOSPHATEMIA

In early stages of hyperparathyroidism, the serum phosphate level is low. Hypophosphatemia is also present in rachitis, osteomalacia, celiac disease, sprue, during attacks of familial periodic paralysis, and in some cases of hyperinsulinism. The hypophosphatemia of rachitis is accompanied by decreased urinary excretion of phosphate, while in hyperparathyroidism, the urinary phosphate excretion is increased. The diphosphoglyceric acid (ester phosphate) of the erythrocytes decreases markedly in acidosis and rachitis; in the latter condition, the administration of vitamin D raises the ester phosphate to normal. In certain types of severe rickets the erythrocytic adenosine triphosphate behaves similarly. Acid-soluble phosphorus of the general tissues decreases in acidosis.

INCREASED SERUM PHOSPHATASE

Alkaline phosphatase values below 11 units per 100 ml. of serum are of little clinical significance in adults. The serum phosphatase activity is definitely increased in hyperparathyroidism, and in vitamin D deficiency (active rachitis and osteomalacia). The determination of serum phosphatase is a reliable means of detecting rachitis, and of estimating improvement from antirachitic therapy. The administration of parathormone increases phosphatase activity, while vitamin D decreases it; these agents also have opposite influences on the concentration of esterified phosphate in the erythrocytes. Serum phosphatase activity is often increased mark-

edly in carcinomatous metastases to bone, in osteogenic sarcoma, and in polyostotic fibrous dysplasia. The serum phosphatase activity is, therefore, useful in detecting metastases or renewed growth of the tumors. Since increase in serum phosphatase is associated with osteoblastic activity, the phosphatase activity of osteogenic sarcoma tissue is greater than that of normal osseous tissue. In destructive or osteolytic types of sarcoma, the serum phosphatase activity is more nearly normal. Serum phosphatase activity is especially high in Paget's disease (osteitis deformans). In this condition, phosphatase determinations are of especial diagnostic value, because the serum calcium and phosphate are usually normal. A large proportion of patients with sprue, obstructive jaundice, hepatocellular jaundice, and portal cirrhosis, and some cases of hepatic carcinoma and biliary fistula, show increased serum phosphatase activity. There is no increase in hemolytic jaundice, or in congenital atresia of the bile duct. Slight increases are found in renal rickets, multiple myeloma, Boeck's sarcoid, and, occasionally, in hyperthyroidism. The acid phosphatase of blood is increased in prostatic carcinoma with skeletal metastases. Estrogen therapy and castration depress the acid phosphatase activity in this condition, while androgens increase it.

RACHITIS (RICKETS); OSTEOMALACIA

In adults, vitamin D deficiency prevents the union of fractures; and severe avitaminosis D is manifested as osteomalacia, characterized by soft bones and marked deformations. In oriental countries, osteomalacia develops rather frequently in pregnant women. When the calcium intake of the pregnant woman is inadequate, the mineral requirement of the fetus is satisfied at the expense of the maternal supply.

Rachitis is a disease of infancy and childhood, caused by deficient vitamin D, calcium or phosphate intake, or insufficient exposure to ultraviolet light. It is characterized by a decreased calcification of the osteoid tissue, skeletal weakness, and deformity. The proliferating cartilage fails to ossify at the provisional zone of calcification and the metaphyses enlarge. Rachitis is, therefore, accompanied by enlargement of the wrists, knees and ankles, contraction of the chest, beading of the ribs, bowing of the legs, and malformation of the spine. Body growth and development are inhibited, and if the disease is long continued, dwarfism results. Phosphatase is not deficient in the zone of hypertrophic cartilage cells, but the esterified phosphate is not converted to bone salt at the normal rate. The serum inorganic phosphate and the organic or ester phosphate (diphosphoglycerate) of the erythrocytes are low, while the serum phosphatase activity is increased. The serum calcium level is nearly normal, except in cases complicated by infantile tetany. The calcium and phosphate content of the bone is subnormal, and the magnesium content is high. Muscle and brain calcium decrease markedly in rachitic animals,

but the calcium concentration remains normal in the other soft tissues. Vitamin D deficiency causes poor intestinal absorption of calcium. The increased fecal excretion of calcium phosphate renders the feces slightly alkaline (pH 7.4). The calcium deficiency stimulates parathyroid activity, which lowers the serum phosphate.

When vitamin D is administered to rachitic children, the absorption of calcium and phosphate is stimulated; parathyroid activity is inhibited; the plasma phosphate returns to the normal level; the phosphatase of the serum is lowered, and normal osteoblastic activity and ossification are resumed. Vitamin D shifts calcium and phosphate from the shafts to the growing areas of bone, and thus aids the healing process even with a relatively low mineral intake. Brain calcium is restored much more slowly. Intravenously injected viosterol raises the calcium content of soft tissues more rapidly than does ingested vitamin D. Dihydotachysterol and parathormone elevate the serum calcium and increase the urinary phosphate excretion, but they cannot substitute for vitamin D in the treatment of rickets. This disease can be cured in parathyroidectomized animals. The daily administration of from 1,000 to 1,500 international units of vitamin D (as cod liver oil) is usually sufficient for successful treatment; in resistant rachitis (rachitis hepatica, etc.), much more vitamin D is required.

Excessive intake of such cations as beryllium, magnesium, iron, lead, strontium, and thallium, which precipitate phosphate in the intestinal tract, interfere with phosphate absorption and cause rachitis. Vitamin D administration is relatively ineffective in these experimental conditions, and also in the Fanconi syndrome (intractable hypophosphatemic rachitis accompanied by acidosis and renal glycosuria). Excessive intake of alkali can induce rachitis in animals, while tartaric and citric acids, added to rachitogenic diets, exert protective effects. Excess cereal intake tends to be rachitogenic, owing to the high phytin and low calcium content; a low phosphate intake is also rachitogenic. Rickets, osteomalacia, and osteoporosis can result from the deficient absorption and storage of calcium and vitamin D in steatorrhea, celiac disease, sprue, and hepatic cirrhosis.

OTHER DISEASES OF BONE

In *achondroplasia*, there is a congenital defect in endochondral ossification of the limb bones, while periosteal ossification proceeds actively. The long bones become short, thick, and dense. Achondroplastic dwarfs show characteristic abnormalities of certain facial bones. *Chondrodystrophy* is the result of disturbance in cartilage formation and perichondral ossification. *Osteoporosis*, which occurs in a variety of pathological conditions, represents a reduction in bone volume and in all of the osseous constituents.

Fractures, enlargement of the skull, and deformities of the extremities occur in *osteitis deformans* or *Paget's disease*. There is marked circumscribed

disorganization of bone; giant cell osteoclasts destroy the old bone spicules, loose connective tissue proliferates, and new bone is formed. Serum calcium and phosphate levels are usually normal, but the serum phosphatase activity is increased markedly in advanced cases. Adrenal cortical extract has been used in the treatment of Paget's disease.

The syndrome of *polyostotic fibrous dysplasia* (osteitis fibrosa disseminata) is characterized by regional osseous abnormality, areas of brown pigmentation, normal serum levels of calcium and phosphate, and, at times, by increased phosphatase activity.

Extreme rarefaction and fragility of bones, and a high incidence of fractures, occur in *osteogenesis imperfecta*. This condition represents a congenital abnormality of osteoid tissue and diminished osteoblastic activity. It is not benefited by vitamin D, or by parathormone therapy. Plasma calcium, phosphate and phosphatase concentrations are normal.

The condition known as *osteopetrosis*, or *marble bone disease*, is characterized by increased density of bone, with contraction and obliteration of the haversian systems and marrow spaces. The plasma calcium and phosphate levels are usually normal.

Osteogenic sarcomas are caused by malignant bone-producing cells, which accelerate both formation and destruction of bone in varying degree. The osteoblastic type of sarcoma stimulates active bone formation, and leads to high phosphatase values in both the tumor and the blood plasma. Irradiation, or removal of the tumor, lowers the serum phosphatase activity, while metastasis and resumption of growth increase it. In the osteolytic type of sarcoma, destruction of bone preponderates. Serum phosphatase activity is not increased, and the serum calcium and phosphate levels are essentially normal. Carcinomatous metastases to bone from carcinoma of the adrenal, breast, prostate, or thyroid often raise the serum phosphatase activity, but they produce little change in the serum calcium and phosphate levels. It is the acid phosphatase activity of blood which is increased by metastases from prostatic carcinoma.

The relatively rare tumor, *multiple myeloma*, arises in the bone marrow; it causes overproduction of marrow proteins (Bence-Jones protein), hyperproteinemia, and dissolution and decalcification of bone, by encroachment of the tumor. (See pages 445 and 448 for a discussion of Bence-Jones protein in blood and urine.) Nephrosis or nephritis is frequently a complicating factor. The plasma levels of phosphate and phosphatase are normal, but hypercalcemia is often present.

In *radium poisoning*, the radioactive mineral is deposited in the bones. Bone and bone marrow are constantly bombarded by destructive alpha particles (nucleus of the helium atom with two positive charges). Anemia, bone rarefaction, necrosis and, occasionally, osteogenic sarcoma result. The radium is first deposited in newly formed trabeculae; later, it is gradually mobilized and distributed throughout the bone. Parathormone tends to mobilize radium from bone.

In *hyperthyroidism*, the urinary and fecal excretion of calcium and phosphate is greatly increased, with little change in the serum levels. This disease is accompanied by rapid osteoporosis and generalized rarefaction of bone. The decalcification is not due to parathyroid stimulation or to acidosis, although the latter syndrome causes a marked increase in urinary calcium. In *hypothyroidism*, the excretion of calcium and phosphate is decreased; the serum phosphatase activity is subnormal in hypothyroid children. Extensive resorption of bone has been noted in *sympathoblastoma* (tumor of the adrenal medulla). The administration of *estradiol* to normal animals causes proliferation of osteoblasts and an increase in the density of bone; this estrogen has been employed to counteract osteoporosis following the menopause. Epiphyseal abnormalities, decreased calcification and delayed healing of fractures occur in *vitamin C deficiency*; the plasma levels of calcium and phosphate are normal.

METASTATIC OR PATHOLOGICAL CALCIFICATION

In *calcinosis*, calcium phosphate and calcium carbonate are deposited in epithelial, interstitial and connective tissue cells in the proportions found in bone salt. In *calcinosis circumscripta*, the deposition is limited to the skin and subcutaneous connective tissue. Ulcers often form about the nodular deposits, and calcinosis of superficial tissues is, at times, associated with *scleroderma* (induration caused by increased intercellular collagenous deposits). In *calcinosis universalis*, the deposits involve the skin, subcutaneous tissue, fascia, nerve sheaths and the coats, septa, origins and insertions of muscles. Calcification which chiefly involves the interstitial muscle tissue is termed *myositis ossificans*. Calcinosis universalis usually appears in childhood. In *calcinosis circumscripta*, the calcium deposits can undergo gradual resorption, while in *calcinosis universalis*, they progress and ultimately interfere with muscle and joint functions, and lead to contractures. Calcinosis patients have a marked tendency to retain excessive quantities of calcium and phosphate; the serum levels of these minerals are normal. Ketogenic low calcium diets, and the administration of ammonium chloride or phosphate, have been used therapeutically to delay the pathological calcification.

Toxic doses of vitamin D, dihydrotachysterol and parathormone, and extensive destruction of bone, lead to pathological *metastatic calcification* and necrosis of soft tissues, and to formation of urinary calculi. The organs which most commonly undergo metastatic calcification are the arteries, cartilages, and such acid-excreting organs as the kidneys, stomach, and lungs. Slight alkalinity and metabolic inactivity, or ischemia, of tissues facilitate the deposition of calcium phosphate and calcium carbonate, while acidity favors reabsorption. Other predisposing factors to metastatic calcification include high serum levels of calcium or phosphate, and high phosphatase activities of tissues. In long-continued severe

hypervitaminosis D, calcium and phosphate are withdrawn from the bone shafts, and these minerals are partly deposited in the epiphyses and soft tissues, and are partly excreted. Diarrhea develops, and the animals lose weight and die. *Dystrophic calcification* occurs in necrotic, devitalized, or chronically inflamed tissues, infarcts, and areas of hyaline or fatty degeneration.

PATHOLOGICAL SERUM MAGNESIUM LEVELS

Serum magnesium is often slightly elevated in chronic glomerulonephritis, hyperparathyroidism, puerperal paralysis of animals, and, at times, in infantile tetany. It is sometimes decreased in rachitis and in the grass tetany of cattle. Low magnesium intake causes magnesium tetany, convulsions, and death in rats and dogs. In this condition, the serum calcium is normal, but the mineral accumulates in the kidney. Combined deficit of calcium and magnesium does not cause tetany. The reported decreases in serum magnesium during severe epileptic convulsions may be secondary to active muscular contractions. The small fraction of serum magnesium which is bound to protein increases in hyperthyroidism, and decreases in hypothyroidism.

PATHOLOGICAL SERUM SULFATE LEVELS

The inorganic sulfate of serum increases with the non-protein nitrogen and the inorganic phosphate in late stages of nephritis.

IRON METABOLISM IN ANEMIAS

The iron requirement is highest during infancy, early childhood, adolescence, and pregnancy; and hypochromic anemia is most common at these times. The iron reserve is low in twins, premature infants, the offspring of iron-deficient mothers, and infants with low birth weight. Postnatal causes of low iron reserve include malnutrition, inadequate iron intake, infections and poor absorption due to diarrhea or achlorhydria. *Hyposideremia*, or low plasma iron level, accompanies idiopathic steatorrhea, iron deficiency or hypochromic anemias, and anemias which are characterized by rapid hemoglobin regeneration (posthemorrhagic anemia, and remission of pernicious anemia). *Hypersideremia*, or increased plasma iron concentration, occurs in acute hepatitis, anemia due to pyridoxin deficiency, aplastic anemia, relapse of pernicious anemia, and, at times, in hemolytic anemia. The iron index (mg. per cent iron in whole blood ÷ red cell count in millions) is high in macrocytic hyperchromic anemia, and low in microcytic hypochromic anemia. The non-hemoglobin iron of erythrocytes (from 2 to 10 per cent of the total iron) increases in aplastic and pernicious anemias, and in some anemias resulting from chronic hemorrhage and nutritional deficiency. The general chemistry and

metabolism of anemias and polycythemias have been discussed on pages 549 to 554.

HEMOCHROMATOSIS

This disease is characterized by marked deposition in tissues of a brown inorganic iron compound, hemosiderin, and an iron-free pigment, hemofuscin (which is related to melanin, and the brown pigment of atrophy and old age). The pigmentation is prominent in the liver, pancreas, kidneys, skin, heart, thyroid, salivary glands, and lymph nodes. Other features of hemochromatosis are hypertrophic cirrhosis, bronze pigmentation of the skin, diabetes mellitus (degenerative changes in the pancreas), and sexual hypoplasia. Hemosiderin gives the Prussian blue reaction, which can be used for its microscopic detection in tissues. In hemochromatosis, the deposition of hemosiderin is so extensive that the body may contain from four to ten times the normal quantity of total iron. There is no change in the iron concentration of plasma or whole blood, unless anemia is present. As the disease progresses, the diabetic symptoms and skin pigmentation appear; this syndrome is termed "bronze diabetes." The spleen and salivary glands undergo extensive fibrosis. Some calcium is deposited with the hemosiderin; the liver and other tissues show an accumulation of copper. Hemochromatosis exhibits familial tendencies; it is about twenty times as common in males as in females. The disease usually requires from four to five decades for full development and for extensive hemosiderin deposition.

PATHOLOGICAL BLOOD COPPER LEVELS

The blood copper concentration is increased by accelerated erythropoiesis, and by erythrocyte destruction (polycythemia and experimental hemolytic anemia). It is decreased in severe experimental copper deficiency.

PATHOLOGICAL BLOOD IODINE LEVELS

The clinical value of blood iodine determinations is limited by technical difficulties. The blood iodine concentration is increased in pregnancy, hepatic and biliary tract disease, and in the early stages of hyperthyroidism. It falls to normal values in late hyperthyroidism after prolonged depletion of the body iodine, also after thyroidectomy. The administration of iodine or iodide in severe hyperthyroidism causes a temporary increase in the inorganic and a decrease in the organic fraction of the blood iodine. The blood iodine level is low in hypothyroidism.

THYROID DISEASES

The iodine of the thyroid gland is contained in the thyroglobulin "colloid" of the follicles. It is present in the globulin as thyroxine and

diiodotyrosine units, both calorigenically active (even though free diiodotyrosine is not). The iodine content of the thyroid is diminished in *simple or colloid goiter*, a condition which is caused by inadequate iodine intake, and which is endemic to areas with low iodine content in the soil, water and plants. As the gland becomes deficient in iodine, it undergoes hyperplasia. This enlargement is usually associated with a decrease in the concentration of iodine in the gland to less than 50 per cent of the normal value. It is followed by involution and increase in the colloid and total iodine of the gland. The concentration of iodine remains subnormal, and the thyroglobulin from colloid goiters contains a much smaller percentage of iodine than does the normal hormone. Both the thyroxine and diiodotyrosine content of the thyroglobulin are low; but the decrease in thyroxine is most pronounced. At times, hyperplasia of the thyroid is followed by exhaustion atrophy. The clinical symptoms of colloid goiter are usually slight, unless the gland enlarges sufficiently to cause pressure. The administration of small quantities of iodide is an established prophylactic and arrestive treatment of simple goiter.

In simple goiter, the thyroid is usually able to supply enough thyroglobulin to prevent the appearance of hypothyroid symptoms, while in *cretinism* of children and *myxedema* of adults, it becomes atrophic, and the parenchyma and colloid largely disappear. These hypothyroid conditions cause mental sluggishness, dry and scaly skin, subnormal temperature, low basal metabolic rate, increased body weight, lipemia, hypercholesterolemia, retention of creatine, calcium, and phosphate, diminution in urea clearance, and release of thyrotropic hormone. There is osseous retardation in cretinism, and a tendency toward subnormal serum phosphatase activity. A myxedematous swelling associated with increased protein content of the extracellular tissue fluids is characteristic of myxedema. The blood iodine falls below normal, the chief decrease being in the alcohol insoluble organic fraction. Administered iodide is assimilated slowly from the blood, and an abnormally large proportion is excreted. Pituitary thyrotropic extracts become inactive on prolonged administration, and in many hypothyroid patients they do not stimulate thyroglobulin synthesis effectively (page 689). Substitution therapy with thyroid is the basis of successful treatment. Iodized casein (which contains as much as 0.4 per cent thyroxine) may be substituted for desiccated thyroid in the control of hypothyroidism. It is important to treat the condition during pregnancy in order to avoid congenital hypothyroidism in the offspring. Prolonged administration of thiouracil, thiourea, or thiocyanate can produce cretinism or myxedema in experimental animals.

In *hyperthyroidism*, the gland is hyperactive; it assimilates iodide faster and synthesizes thyroglobulin more rapidly than the normal gland, and thus raises the basal metabolic rate. All fractions of the thyroid iodine are subnormal; the thyroxine component is especially low. The blood iodine is frequently elevated, especially the alcohol insoluble organic fraction, and

there is increased excretion of iodine in the urine, feces, and sweat. The longer the condition exists, the greater is the tendency for the blood iodine to recede, owing to depletion of the hormone. When Lugol's solution or iodide is administered to hyperthyroid patients, there is a temporary remission of symptoms, decrease in the basal metabolic rate, increase in the iodine content of the gland, and elevation of the thyroxine content of the thyroglobulin. This remission is caused by inhibition of thyroid secretion (antithyrotropic action). Iodine (or iodide) and thiamin medication are used before and after thyroid surgery. Adequate ascorbic acid should be administered to hyperthyroid patients, since this vitamin is rapidly depleted in the liver, adrenal glands, and other tissues. The daily administration of 0.1 to 1 gm. thiouracil is an established medical treatment for thyrotoxicosis and for its preoperative management. The dosage must be ascertained for the individual patient. Thiouracil depresses thyroid assimilation of iodide and synthesis of thyroglobulin. It lowers the basal metabolic rate, and ameliorates other symptoms of hyperthyroidism. The anterior pituitary gland responds by increasing its thyrotropin secretion, which causes a compensatory hyperplasia and hypertrophy of the thyroid, and an increase in its rate of respiration. The effects of thiouracil and related substances (page 612) can be counteracted by thyroxine, but not by iodide. Iodide can prevent the thyroid effects of thiocyanate. One third or more of administered thiouracil is destroyed in the gastro-intestinal tract and tissues of the patient; the remainder is excreted in the urine. Thiouracil must be used with caution in pregnant patients since it passes the placenta readily. The blood lipid and cholesterol content is lowered in hyperthyroidism, particularly in severe exophthalmic goiter. About one half the cases of hyperthyroidism show decreased glucose tolerance, lowered liver glycogen, and reduced hepatic function. The muscular weakness is accompanied by creatinuria and impairment of the phosphocreatine mechanism. The excretion of nitrogen, calcium, and phosphate is increased, and osteoporosis results. The low thyrotropin blood level in hyperthyroid patients is attributed to rapid inactivation of the hormone by the abnormally active thyroid tissue. Other phases of thyroid diseases are considered on pages 707 to 710.

BROMISM

Prolonged or excessive ingestion of bromide can induce chronic toxicity, characterized by skin eruptions, catarrhal changes in mucous membranes, decreased intellectual acuity, and other central nervous disturbances. Such intoxication is likely to occur when the serum bromide exceeds 150 mg. per cent. When 40 per cent of the blood chloride is replaced by bromide, death results. A low chloride intake accentuates the effects of bromide administration, while ingestion of excess sodium chloride and administration of desoxycorticosterone aid replacement of bromide by

chloride in body fluids, and stimulate bromide elimination. Since the kidneys excrete bromide more slowly than chloride, excessive bromide intake can readily produce edema.

FLUOROSIS; MOTTLED ENAMEL

When the food of mammals contains more than 10 mg. per cent of fluoride, or the drinking water more than 0.1 mg. per cent, fluorosis occurs. Acute fluoride poisoning can prove fatal, partly through lowering of the serum calcium concentration. Calcium retention and blood phosphatase are diminished and growth is retarded. The fluoride enters into the lattice of bone salt, and causes alterations in the size, shape, and composition of bones. Osteoblastic activity is disorganized; calcification is excessive and irregular, and the calcium : phosphorus ratio of bone is lowered. Adult animals develop an osteosclerosis with thickening of the trabeculae, periosteal depositions, and narrowing of the marrow cavity. In young animals, excessive fluoride intake causes osteoporosis.

The most common effect of fluoride excess in humans is mottled enamel of teeth, characterized by opaque, brittle enamel which turns brown. This condition is endemic to areas which have excess fluoride in the water supply. Mottled enamel is most frequent in the permanent teeth of persons whose daily fluoride intake was above 0.1 mg. per kg. of body weight during the first eight years of childhood. A trace of dietary fluoride (0.5 to 1 mg. per day) during this period is considered necessary for normal enamel formation and for prophylaxis of dental caries. The incidence of dental caries in children appears to vary inversely with the fluoride concentration in the water supply. Enamel of carious teeth has a subnormal fluoride content.

BIBLIOGRAPHY

METABOLISM OF MINERALS

General

- American Medical Association. Handbook of Nutrition. 1943.
- BRIDGES, M. A. Dietetics for the Clinician. Ed. 4. Philadelphia, Lea and Febiger, 1941.
- FENN, W. O. Electrolytes in muscle. *Physiol. Rev.*, 16 : 450, 1936.
- HASTINGS, A. B. The electrolytes of tissues and body fluids. *Harvey Lect.*, 36 : 66, 1940-41.
- KURBATOV, J. D., and POOL, M. L. Radioactive isotopes for the study of trace elements in living organisms. *Chem. Rev.*, 32 : 231, 1943.
- NEEDHAM, J. Chemical Embryology. New York, Macmillan, 1931. (3 vol.)
- SHOHL, A. T. Mineral Metabolism. New York, Reinhold, 1939.
- STEARN, G. The mineral metabolism of normal infants. *Physiol. Rev.*, 19 : 415, 1939.

WAY, S. C., and MEMMESHEIMER, A. M. Sweat. *Arch. Dermat. & Syph.*, 41 : 1086, 1940.

Urine Secretion

SHANNON, J. A. Renal tubular excretion. *Physiol. Rev.*, 19 : 63, 1939.

SMITH, H. W. The Physiology of the Kidney. London, Oxford Univ. Press, 1937.

SMITH, H. W. Studies on the Physiology of the Kidney. Lawrence, Extension Division Univ. of Kansas, 1939.

VAN SLYKE, D. D. Renal mechanisms controlling composition of body fluids. *Chem. Rev.*, 26 : 105, 1940.

WINTON, F. R. Physical factors in the activities of the mammalian kidney. *Physiol. Rev.*, 17 : 408, 1937.

Water; Alkali Cations; Chloride

DRINKER, C. K., and YOFFEY, J. M. Lymphatics, Lymph and Lymphoid Tissue. Cambridge, Harvard Univ. Press, 1941.

FENN, W. O. The role of potassium in physiological processes. *Physiol. Rev.*, 20 : 377, 1940.

GAMBLE, J. L. Chemical Anatomy, Physiology and Pathology of Extracellular Fluid. Cambridge, Harvard Univ. Press, 1942.

INGRAM, W. R. Relations of the Hypophysis to Water Exchange. *Cold Spring Harbor Symp. Quant. Biol.*, 3 : 381, 1937.

IRVING, L., and MANERY, J. F. Significance of the chlorides in tissues and animals. *Biol. Rev. Cambridge Phil. Soc.*, 11 : 287, 1936.

NEWBURGH, L. H., and JOHNSTON, M. W. The insensible loss of water. *Physiol. Rev.*, 22 : 1, 1942.

PETERS, J. P. Body Water, the Exchange of Fluids in Man. Springfield, Thomas, 1935.

PETERS, J. P. Water exchange. *Physiol. Rev.*, 24 : 491, 1944.

Calcium; Magnesium; Phosphate; Phosphatase; Sulfate

ANNERSTEN, S. Osteogenesis and the biochemistry of fracture callus. *Acta chir. Scandinav.*, Suppl. 60, 1940.

DUCKWORTH, J. Magnesium in animal nutrition. *Nutrition Abstr. & Rev.*, 8 : 841, 1939.

GARDNER, W. U., and PFEIFFER, C. A. Influence of estrogens and androgens on the skeletal system. *Physiol. Rev.*, 23 : 139, 1943.

GUEST, G. M., and RAPOPORT, S. Organic acid-soluble phosphorus compounds of the blood. *Physiol. Rev.*, 21 : 410, 1941.

HARRIS, H. A. Bone Growth in Health and Disease. London, Oxford Univ. Press, 1933.

HUGGINS, C. Composition of bone and the function of bone cells. *Physiol. Rev.*, 17 : 119, 1937.

LOGAN, M. A. Recent advances in the chemistry of calcification. *Physiol. Rev.*, 20 : 522, 1940.

REED, C. I., et al. Vitamin D. Chicago, Univ. of Chicago Press, 1939.

REID, M. E. Interrelations of calcium and ascorbic acid to cell surfaces and inter-cellular substances. *Physiol. Rev.*, 23 : 76, 1943.

- ROBERTSON, J. D. The function and metabolism of calcium in the invertebrata. *Biol. Rev. Cambridge Phil. Soc.*, 16 : 106, 1941.
- SCHMIDT, C. L. A., and GREENBERG, D. M. Calcium, magnesium and phosphorus in the animal organism. *Physiol. Rev.*, 15 : 297, 1935.
- SCHOUR, I. Calcium metabolism and teeth. *J. A. M. A.*, 110 : 870, 1938.
- SMITH, P. K., *et al.* Pharmacological actions of parenterally administered magnesium salts. *Anesthesiology*, 3 : 323, 1942.
- STADIE, W. C. The relation of insulin to phosphate metabolism. *Yale J. Biol. & Med.*, 16 : 539, 1944.

Trace Metals

- ELVEHJEM, C. A. Biological significance of copper. *Physiol. Rev.*, 15 : 471, 1935.
- HAHN, P. F. The metabolism of iron. *Medicine*, 16 : 249, 1937.
- HEGSTED, D. M., *et al.* The biological and medical properties of zinc and zinc compounds. *U. S. Public Health Repts.*, Suppl. 179, 1945.
- HENDERSON, V. E., and LUCAS, G. H. W. Absorption of iron. *Am. J. Digest. Dis. & Nutrition*, 11 : 244, 1944.
- HILL, W. R., and PILLSBURY, D. M. Argyria. The Pharmacology of Silver. Baltimore, Williams and Wilkins, 1939.
- KEHOE, R. A., *et al.* Trace metals in normal biological material. *J. Nutrition*, 20 : 85, 1940.
- MINOT, A. S. Physiological effects of small amounts of lead. *Physiol. Rev.*, 18 : 554, 1938.
- OETTINGEN, W. F. VON. Manganese, its distribution, pharmacology and health hazards. *Physiol. Rev.*, 15 : 175, 1935.
- SCHARRER, K. Biochemie der Spurenelemente. Berlin, P. Parey, 1941.
- SCHULTZE, M. O. Metallic elements and blood formation. *Physiol. Rev.*, 20 : 37, 1940.
- SHILS, M. E., and MCCOLLUM, E. V. The trace elements in nutrition. *J. A. M. A.*, 120 : 609, 1942.
- UNDERWOOD, E. J. Significance of trace elements in nutrition. *Nutrition Abstr. & Rev.*, 9 : 515, 1940.
- VAHLQUIST, B. C. Serum iron. *Acta Paediat.*, 28 : Suppl. V, 1941.

Trace Anions

- ELMER, A. W. Iodine Metabolism and Thyroid Function. London, Oxford Univ. Press, 1938.
- KING, E. J., and BELT, T. H. The physiological and pathological aspects of silica. *Physiol. Rev.*, 18 : 329, 1938.
- SALTER, W. T. The Endocrine Function of Iodine. Cambridge, Harvard Univ. Press, 1940.
- SOSKIN, S., and LEVINE, R. Recent advances in physiology of the thyroid and their clinical application. *Arch. Int. Med.*, 74 : 375, 1944.

PATHOLOGY

General

- CORNBLEET, T. Mineral metabolism and the skin. *Urol. & Cutan. Rev.*, 45 : 451, 1941.

- COWDRY, E. V. *Problems of Aging*. Ed. 2. Baltimore, Williams and Wilkins, 1942.
- FOLLIS, R. H., JR. Pathologic effects produced by deficiency of single elements. *Arch. Path.*, 34 : 451, 1942.
- KUGELMASS, I. N. *Newer Nutrition in Pediatric Practice*. Philadelphia, Lippincott, 1940.

Dehydration; Shock; Diabetes Insipidus; Edema

- BLALOCK, A. Peripheral circulatory failure. *Am. Heart J.*, 23 : 147, 1942.
- COLLER, F. A., and MADDOCK, W. G. Water and electrolyte balance. *Surg. Gynec. & Obst.*, 70 : 340, 1940.
- DARROW, D. C. The treatment of dehydration, acidosis and alkalosis. *J. A. M. A.*, 114 : 655, 1940.
- FISHER, C., *et al.* Diabetes Insipidus and the Neurohormonal Control of Water Balance. Ann Arbor, Edwards, 1938.
- GIBSON, J. G., 2nd. The clinical significance of the blood volume. *Ann. Int. Med.*, 14 : 2014, 1941.
- HARKINS, H. N. Recent advances in the study and management of traumatic shock. *Surgery*, 9 : 231, 447, 607, 1941.
- LUISADA, A. The pathogenesis of paroxysmal pulmonary edema. *Medicine*, 19 : 475, 1940.
- MARVIN, H. M. The therapy of dropsy. *J. A. M. A.*, 114 : 757, 1940.
- MOON, V. H. Shock: Its Dynamics, Occurrence and Management. Philadelphia, Lea and Febiger, 1942.
- SCUDDER, J. Shock; Blood Studies as a Guide to Therapy. Philadelphia, Lippincott, 1940.

Pathology of Alkali Metals and Chloride (Addison's Disease; Familial Paralysis; Heat Cramps)

- LOEB, R. F. Adrenal cortical insufficiency. *J. A. M. A.*, 116 : 2495, 1941.
- LOEB, R. F. The adrenal cortex and electrolyte behavior. *Bull. New York Acad. Med.*, 18 : 263, 1942.
- LYALL, A. Pathology of chloride metabolism in man. *Brit. M. J.*, II : 760, 1939.
- MADDOCK, W. G., and COLLIER, F. A. Sodium chloride metabolism of surgical patients. *Ann. Surg.*, 112 : 520, 1940.
- TALBOT, J. H. Heat cramps. *Medicine*, 14 : 323, 1935.
- TALBOT, J. H. Periodic paralysis. *Medicine*, 20 : 85, 1941.
- TALBOT, J. H., and SCHWAB, R. S. Biochemistry and therapeutics of potassium salts. *New England J. Med.*, 222 : 585, 1940.

Rachitis; Osteomalacia; Tetany

(See references to Avitaminosis D, page 678.)

- American Medical Association. *The Vitamins*. Chicago, 1939.
- MCLEAN, F. C. Activated sterols in the treatment of parathyroid insufficiency. *J. A. M. A.*, 117 : 609, 1941.
- PARK, E. A. The therapy of rickets. *J. A. M. A.*, 115 : 370, 1940.

Hyperparathyroidism; Renal Rickets

- ALBRIGHT, F. The parathyroids—physiology and therapeutics. *J. A. M. A.*, 117 : 527, 1941.
- CHARNOCK, D. Renal rickets. *J. Urol.*, 44 : 850, 1940.
- SNAPPER, I. Medical Clinics on Bone Diseases. New York, Interscience Pub., 1943.

Miscellaneous Pathology of Calcium, Magnesium, and Phosphate

(See references to Dental Pathology, page 173.)

- BARR, D. P. Pathological calcification. *Physiol. Rev.*, 12 : 593, 1932.
- BROOKS, W. D. W. Calcinosis. *Quart. J. Med.*, 3 : 293, 1934.
- CORNLEET, T., and STRUCK, H. C. Calcium metabolism in scleroderma. *Arch. Dermat. & Syph.*, 35 : 188, 1937.
- EVANS, R. D. Radium poisoning. *Am. J. Pub. Health*, 23 : 1017, 1933.
- HAURY, V. G. Serum magnesium in health and disease. *J. Lab. & Clin. Med.*, 27 : 1361, 1942.
- JAFFE, H. L., and BODANSKY, A. Diagnostic significance of serum alkaline and acid phosphatases in relation to bone disease. *Bull. New York Acad. Med.*, 19 : 831, 1943.
- JONES, R. W., and ROBERTS, R. E. Calcification, decalcification and ossification. *Brit. J. Surg.*, 20 : 461, 1933-34.
- KATO, K. Calcinosis. In Practice of Pediatrics. Vol. III. Hagerstown, Prior, 1937.
- LAMB, F. H., and JACKSON, R. L. Osteopetrosis (marble bone disease). *Am. J. Clin. Path.*, 8 : 255, 1938.
- MAIR, W. F. Myositis ossificans progressiva. *Edinburgh M. J.*, 39 : 13, 1932.
- SNAPPER, I. Medical Clinics on Bone Diseases. New York, Interscience Pub., 1943.
- SUNDERMAN, F. W. Significance and interpretation of phosphatase in disease. *Am. J. Clin. Path.*, 12 : 404, 1942.
- TOMEY, E. V. Calcium therapy in dental practice. *Brit. Dent. J.*, 63 : 514, 1937.

Hemochromatosis; Iron Deficiency

(See references to Anemias, page 566.)

- BUTT, H. R., and WILDER, R. M. Hemochromatosis. *Arch. Path.*, 26 : 262, 1938.
- FOWLER, W. M., and BARER, A. P. Iron metabolism and its relation to anemia therapy. *Ann. Int. Med.*, 14 : 378, 1940.
- SHELDON, J. H. Hemochromatosis. London, Oxford Univ. Press, 1935.

Pathology of Iodine; Thyroid Diseases

- CROTTI, A. Diseases of the Thyroid, Parathyroids and Thymus. Ed. 3. Philadelphia, Lea and Febiger, 1938.
- ELMER, A. W. Iodine Metabolism and Thyroid Function. London, Oxford Univ. Press, 1938.
- HERTZLER, A. E. Diseases of the Thyroid Gland. New York, Hoeber, 1941.
- MCCLENDON, J. F. Iodine and the Incidence of Goitre. Minneapolis, Univ. of Minnesota Press, 1939.

MEANS, J. H. *The Thyroid and Its Diseases*. Philadelphia, Lippincott, 1937.

THOMPSON, W. O. Thyroid dysfunctions and their treatment. *J. A. M. A.*, 117: 441, 1941.

Miscellaneous

(See references to Cerebrospinal Fluid, page 349.)

American Association for the Advancement of Science. Fluorine and Dental Health. Lancaster, Science Press, 1942.

GOLDSTEIN, H., and MCFARLAND, R. A. Biochemistry of epilepsy. *Am. J. Psychiat.*, 96 : 771, 1940.

GREENWOOD, D. A. Fluoride intoxication. *Physiol. Rev.*, 20 : 582, 1940.

KING, E. J., and BELT, T. H. Physiological and pathological aspects of silica. *Physiol. Rev.*, 18 : 329, 1938.

LANZA, A. J. *Silicosis and Asbestosis*. London, Oxford Univ. Press, 1939.

MOXON, A. L., and RHIAN, M. Selenium poisoning. *Physiol. Rev.*, 23 : 305, 1943.

VOLKER, J. F., and BIBBY, B. G. Action of fluorine in limiting dental caries. *Medicine*, 20 : 211, 1941.

CHAPTER IX

VITAMINS AND AVITAMINOSES



"There can be no intelligent trial in any art without rational ideas as to what we may expect to achieve; we must reflect on the consequence of doing this or that." — MORRIS R. COHEN

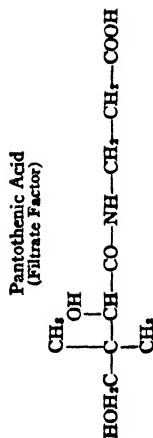
INTRODUCTION

Vitamins are organic dietary substances, small quantities of which are necessary for normal cellular function.¹ They are essential nutritional factors for animals, plants, and heterotrophic micro-organisms, although some of the vitamins are required by relatively few species.

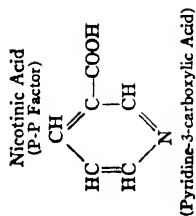
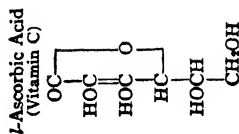
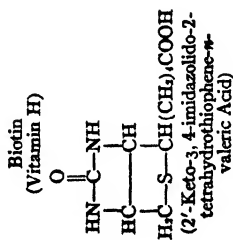
Vitamins are necessary for the promotion of growth in young animals. They exert very powerful influences on metabolism, and their biological activities are partly traceable to coenzymic effects. Several of the vitamins have been identified as units of the prosthetic radicals of intracellular enzymes (pages 101 to 105). Vitamins A, D, E, and K are fat soluble; the others are water soluble. The fat-soluble vitamins are multiple in nature; the related homologues which exert similar physiological activity are differentiated by subnumerals, as for example, vitamins A₁ and A₂. Subnumerals in the vitamin B series do not refer to homologues, but indicate very different components of the vitamin B complex. The chemistry of vitamins has been discussed in preceding chapters; vitamin formulae are reproduced in Table 103. Nomenclature, unit equivalents, and human requirements are recorded in Table 104; and the distribution in vitamin-rich foods, in Table 105. Earlier estimates of human vitamin requirements have been increased gradually, with recognition of the symptoms of slight deficiencies. Variation in the intestinal bacterial synthesis of vitamin K and vitamins of the B complex is a factor which affects the individual dietary requirements. In general, the fat-soluble vitamins are stored to a greater extent than the water-soluble ones.

Avitaminoses, or vitamin deficiencies, can be produced by inadequate diets; deficient absorption accompanying vomiting, diarrhea, steatorrhea, and gastro-intestinal diseases; increased demand during rapid growth, pregnancy, lactation, and profuse sweating; and accelerated vitamin ca-

¹ By custom, the essential amino acids are not included in this category, and the designation of essential fatty acids as vitamins is not approved officially.



(α,γ-Dihydroxy-β,β-dimethylbutyryl-β'-alanide)



(Pyridine-3-carboxylic Acid)

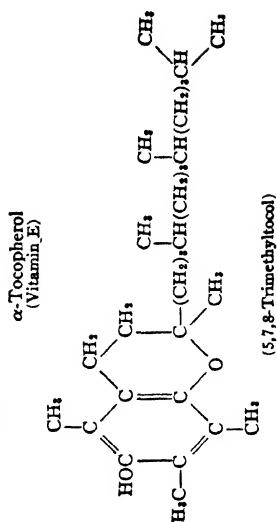
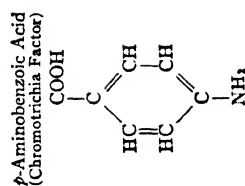
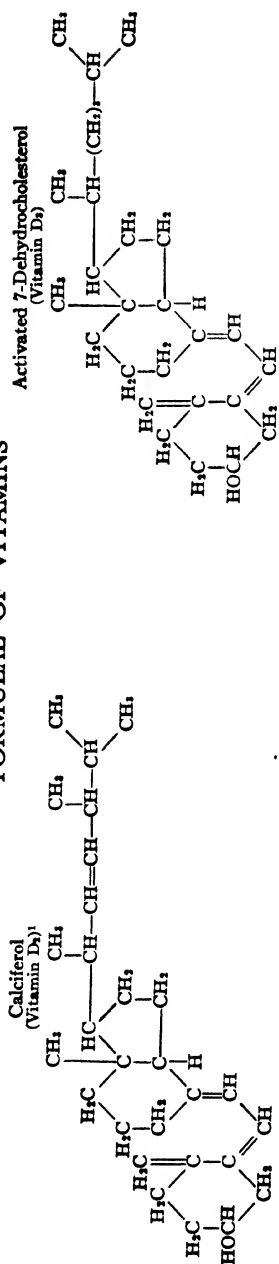
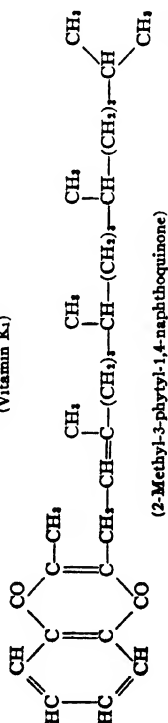
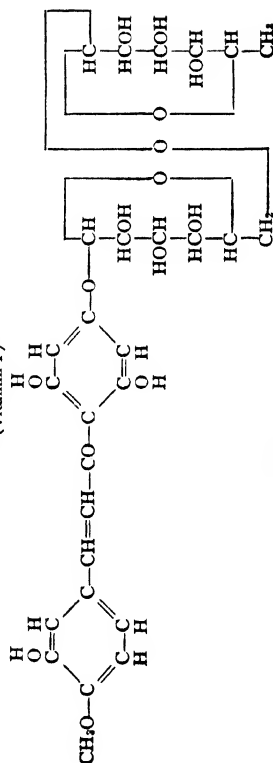
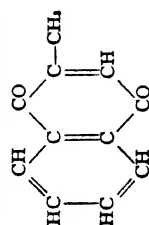


TABLE 103 (Cont.)
FORMULAE OF VITAMINS α -Phylloquinone
(Vitamin K₁)Hesperidin Chalcone
(Vitamin P)(Hesperetin- β -rutinoside)

2-Methyl-1,4-Naphthoquinone :

¹ See Table 36, page 194, for steride formulae.² Synthetic substitute for vitamin K.

tabolism in fever, hyperthyroidism, infections, and so forth. The avitaminoses, and the diseases which are secondary to these deficiencies, prevail in winter and spring in the northern hemisphere, owing to a yearly cycle in the vitamin content of foods. A general symptom of avitaminoses in children and young animals is delayed or stunted growth. Multiple deficiencies, especially of the B complex vitamins, are not infrequent in the clinic. In such instances, cure of a prevailing deficiency can unmask or activate the other avitaminosis.

Vitamins exhibit a wide margin of therapeutic safety; the toxic doses of these substances are hundreds to thousands of times the therapeutic and prophylactic doses. The minimal therapeutic dose of a vitamin is usually from five to ten times the quantity necessary to prevent deficiency disease. Parenteral administration of vitamins is much more effective than oral dosage in raising the blood and tissue vitamin levels; certain therapeutic effects are obtained only by parenteral administration.

VITAMIN A

Vitamins of the A series are animal products, which are formed from carotenoid precursors of plant origin. These vitamins and provitamins are fat-soluble β -ionone derivatives, whose chemistry has been discussed on pages 205 and 206. Vitamins A occur in animal fats and oils as fatty acid esters, while in saponified commercial preparations they are present as the free alcohols. Vitamin A₁ is found in the tissues of all animals, but vitamin A₂ is a specific product of the hepatic metabolism of fishes. In the tissues of fresh water fishes, vitamin A₂ preponderates over vitamin A₁. The carotenoid provitamins include the hydrocarbons, α -, β -, and γ -carotenes, cryptoxanthine, and echinenone. Liver oils contain kitols (divitamins A) which can be converted to active forms by heating. The plant provitamins are usually found in association with chlorophyll, and they tend to accumulate in green and yellow plant tissues. The vitamins and provitamins A are fairly thermostable. They are not inactivated appreciably by ordinary cooking and canning processes, or by storage of canned foods. Drastic or prolonged heating (roasting) and dehydration (sundrying) cause oxidative destruction of vitamin A.

The rat-growth method of assay is the official procedure for evaluating the vitamin A activity of foods. Activities so determined do not have absolute value in terms of vitamin A concentrations, since the vitamin A potency of foods depends partly on the efficiency of intestinal absorption, and the conversion of provitamins to vitamins, in the test animal. The biological activities of vitamins A₁ and A₂ are considered identical; β -carotene is only from 30 to 50 per cent as active as the vitamins A (Table 104). Esters of vitamin A have the same activity as equivalent quantities of the free vitamin. Important dietary sources of vitamin A include liver, egg yolk, butter, and cream; the principal dietary sources of

TABLE 104

NOMENCLATURE, UNIT EQUIVALENTS, AND HUMAN REQUIREMENTS OF VITAMINS ^{1, 2}

NAME		UNIT EQUIVALENT	APPROXIMATE DAILY ALLOWANCES FOR NORMAL HUMANS ³ (Units, Except as Noted)		
Biological	Chemical		Infant	Child	Adult
A (antixerophthalmic)	Axerophthol ⁴	0.6 γ β -Carotene ⁵	1500	4000 \pm 2000	5000 \pm 500
B ₁ (antineuritic)	Thiamin	3.0 γ Thiamin hydrochloride ⁶	170	580 \pm 250	630 \pm 30
B ₂ (growth)	Riboflavin	2.2 γ Riboflavin ⁷	455	1360 \pm 455	1320 \pm 45
B ₆ (rat antidermatitis)	Pyridoxin		1 mg.	3 mg.	2.9 mg.
C (antiscorbutic)	L-Ascorbic acid	50 γ Ascorbic acid ⁸	600	1500 \pm 500	1500 \pm 100
D (antirachitic)	Calciferol ⁹	0.025 γ Calciferol ⁹	30 mg.	75 mg.	75 mg.
E (antisterility)	α -Tocopherol ¹⁰	1 mg. <i>dl</i> - α -Tocopherol acetate ⁸	400	400 \pm 100	400 \pm 100
F ¹¹	Linoleic and linolenic acids				
G (same as B ₂)					
H (anti-egg white injury)	Biotin ¹²	0.037 γ Biotin methyl ester			
K (antihemorrhagic)	α -Phylloquinone ¹³	1 γ 2-Methyl-1,4-naphthoquinone ¹⁴			
P ¹⁵	Hesperidin chalcone				
Filtrate factor (chick antidermatitis)	Pantothenic acid	14 γ Calcium pantothenate ¹⁶			10 mg.
P-P factor (antipellagra)	Nicotinic acid		5 mg.	17.5 \pm 7.5 mg.	20 \pm 2 mg.
Chromotrichia factor	<i>p</i> -Aminobenzoic acid				

¹ Alphabetical nomenclature applied to unidentified vitamins: B₀, chick antianemia factor, perhaps identical with folic acid; B₃, pigeon maintenance factor; L, lactation factor; factor W, heat-labile rat growth factor.

² Other vitamins of the B complex are listed on page 652.

³ These values are based upon recommendations of the National Research Council.

⁴ The term used in Europe for vitamin A₁; no official name has been adopted in the United States. β -Carotene is a provitamin A.

⁵ The international unit, approximately equal to 0.23 γ of crystalline vitamin A₁.

⁶ The international unit, equal to 2 Chase-Sherman units.

⁷ The Sherman-Bourquin unit.

⁸ The international unit.

⁹ Calciferol is vitamin D₂; no chemical name has been adopted for vitamin D₃.

¹⁰ β - and γ -tocopherols are also active.

¹¹ The term vitamin F is not officially approved.

¹² Also known as coenzyme R.

¹³ Vitamin K₁.

¹⁴ The methylnaphthoquinone unit.

¹⁵ Vitamin nature not proved.

¹⁶ The chick filtrate factor unit; the gram unit equals 80 γ calcium pantothenate.

the carotenoid provitamins are butter and the green leafy and yellow vegetables listed in Table 105. Oleomargarine is frequently fortified with vitamin A by the addition of fish liver oil concentrates. The fish liver oils and carotene preparations are potent therapeutic agents, whose usual vitamin A activities in international units per 100 gm. are as follows:

cod liver oil, from 100,000 to 210,000; the customary solution of carotene in cotton seed oil, 750,000; cod liver oil concentrates, from 1,400,000 to 6,000,000; halibut liver oil, from 4,500,000 to 6,500,000; and equal parts of percomorph and cod liver oils, 6,000,000. The recommended daily allowances for humans are: infants, 1,500 units; growing children, from 2,000 to 6,000 units; normal adults, 5,000 units; pregnant or lactating women, from 6,000 to 8,000 units.

The vitamin A esters of foods are hydrolyzed by esterase in the small intestine; during absorption, the vitamin is re-esterified with fatty acids. Provitamins of plant foods are absorbed much less rapidly and completely than vitamin A, and they require bile salt for their absorption, whereas the vitamin does not. The vitamin A esters are not absorbed unless they are hydrolyzed. Carotene absorption is facilitated by cooking the vegetables which contain it. Absorption of the provitamins is accelerated by the ingestion of unsaturated fats. Liquid petrolatum has an inhibitory effect on carotene absorption. Ordinarily, from 50 to 70 per cent of the dietary carotene is lost in the feces. The antioxidant vitamin, tocopherol, exerts a sparing action on vitamin A by inhibiting its oxidative destruction in the gastro-intestinal tract. The vitamin A esters and provitamins are transported in the blood and lymph. The normal carotene (total carotenoid) concentration of blood is $165 \pm 60 \gamma$ per cent for men, and $185 \pm 75 \gamma$ per cent for women; the corresponding vitamin activities are 125 ± 30 , and 100 ± 20 international units per 100 ml. of blood, respectively. Fetal blood has a lower vitamin A activity and carotenoid concentration. The plasma of the newborn infant contains only half as much vitamin A, and one tenth as much carotene, as adult plasma. There is little increase in the vitamin A activity of blood after an ordinary meal, but the ingestion of a teaspoonful of halibut liver oil more than doubles the activity within two hours. Maximal values are attained in four and seven hours respectively, after the ingestion of large doses of vitamin A and carotene. Normal cerebrospinal fluid contains no vitamin A.

Vitamin A is distributed widely in tissues, but the liver is responsible for 95 per cent of the vitamin A storage of mammals. The provitamins are stored both in the liver and in adipose tissue. Administration of alcohol or carcinogens mobilizes vitamin A from the liver. The liver of a well nourished human adult contains about 10 mg. per cent vitamin A in esterified form (chiefly vitamin A palmitate), while the liver of the newborn child has only from 0.5 to 2.5 mg. per cent. The very limited vitamin A reserve of the fetus and the newborn is partly due to the difficulty with which the vitamin traverses the placenta. A similar limitation of transfer applies to the mammary gland; the administration of cod liver oil to a lactating female does not increase the vitamin A activity of the milk appreciably. Breast milk secreted in early stages of lactation has from five to ten times the vitamin A activity of cow's milk; but as lactation continues, the vitamin A activity of breast milk becomes comparable to that of cow's milk.

TABLE 105

APPROXIMATE VITAMIN CONTENT OF VITAMIN-RICH FOODS¹

VITAMIN A	I. U. PER 100 GM.	THIAMIN	γ PER CENT	RIBOFLAVIN	γ PER CENT
Parsley	30,000	Brewer's yeast (moist)	1,200	Liver	1,700
Spinach	18,000	Brazil nuts, pecans, pork	1,100	Yeast	1,600
Endive	15,000	Peanuts	900	Kidney	1,400
Dandelion greens	12,000	Wheat (whole)	600	Almonds, heart	600
Broccoli, chard	9,000	Heart, oatmeal	550	Eggs, oysters, peanuts	450
Liver	8,000	Kidney, green peas	500	Fortified cereals	330
Peppers	5,000	Fortified cereals	460	Crabmeat, pecans	300
Apricots, lettuce, watercress	4,000	Rye (whole), walnuts	400	Watercress	270
Sweet potatoes	3,500	Egg yolk, lentils, soya beans	370	Broccoli, salmon	225
Egg yolk	2,800	Fortified bread	360	Beans, beef, cow's milk, fortified bread	200
Tomatoes	2,600	Beans, bread (whole wheat), mutton, liver	300	Cheese (cream), codfish, halibut	180
Papaya, pumpkin, squash	2,500	Baker's yeast (moist)	250	Haddock, mutton, papaya, pork, shrimp	160
Butter	2,400	Almonds, cauliflower	225	Green peas	150
Carrots, cheese (cream), rice	2,000	Green asparagus, beef, rye bread, rice (whole)	200	Lobster, trout, sweet potatoes	130
Prunes	1,500	Brains, brussels sprouts, carrots, corn, watercress	180	Asparagus, endive, peppers, rice (whole), tomatoes	120
Green beans, green celery, eggs, kidney, yellow peaches, green peas, tomato juice, walnuts	1,000	Crabmeat, lettuce	160	Cauliflower, coconut, corn	100
		Mushrooms, gooseberries	150		
		Bananas, spinach	135		
		Apples, grapefruit, oranges, potatoes, turnips	120		

TABLE 105 (Cont.)
APPROXIMATE VITAMIN CONTENT OF VITAMIN-RICH FOODS¹

Nicotinic Acid	Mg. Per Cent	Ascorbic Acid	Mg. Per Cent	Vitamin D	I. U. Per 100 Gm.
Liver extract	250-450	Currants (black)	200	Salmon	500
Rice bran	165	Parsley, peppers	150	Egg yolk	325
Yeast (dried)	50	Guavas	125	Butter	80
Kidney, liver	15	Horse radish	100	Cream	50
Yeast (moist), peanuts, chicken	12	Brussels sprouts	90	Fortified milk	40
Pork, lamb	9	Cabbage	80	Liver	30
Beef, heart, veal	7	Broccoli, cauliflower, spinach, strawberries	70	Irradiated milk	15
Brain, soya beans, wheat (whole)	5	Kohlrabi	60	Oysters	5
Pancreas, halibut, trout	4	Gooseberries, grapefruit, huckleberries, lemon juice, orange juice, papaya, sweet potatoes, tangerines, tomatoes, watercress	45	Milk	2
Fortified cereals	3.3	Asparagus, beans, blueberries, lime juice, liver, green peas, pineapple	35		
Fortified bread	1.6	Cantaloupe, celery, currants (red), dandelion greens, honey, turnips	30		
Potatoes, spinach, scallops	1.5				
Bread (whole wheat), rye	1.2				
Corn, egg yolk, oats, haddock	1.0				
Milk	0.1				

¹ The vitamin content of foods varies according to the locality, the season of the year, and the variety or species. The values given represent averages.

Human colostrum has from two to three times the vitamin A activity of early breast milk.

The Kupffer cells of the liver contain an enzyme, carotenase, which oxidizes carotene to vitamin A. β -Carotene can be converted to two mols of the vitamin, while the other provitamins form only one mol each. The *neo* carotene isomers are only one fourth to one half as active as the corresponding natural *all-trans* isomers. The conversion of carotene to vitamin A is inhibited by hepatic disease, diabetes mellitus, and hypothyroidism. The specific physiological functions of vitamin A are:

(a) Maintenance of normal epithelia in the skin, eye, upper respiratory, gastro-intestinal and genito-urinary tracts, also in the ducts and acinar tissues of secretory glands

(b) Maintenance of normal nerve tissue

(c) Promotion of normal growth of bone and of tooth enamel

(d) Participation in the visual cycle of dark adaptation.

The chemistry of rhodopsin and iodopsin, the carotenoid-protein pigments of the rods and cones, has been considered on page 206. Dark adaptation depends on the formation of these photosensitive retinal pigments. The rods are concerned with colorless vision at low light intensity, and the cones with color vision at higher light intensity. The regions of maximal spectral sensibility are 555 $m\mu$ for cones, and 500 $m\mu$ for rods; the cones require fifty to one hundred times more energy than the rods for minimal stimulation. During continued illumination, an equilibrium exists between the destruction and resynthesis of rhodopsin. Quanta of light react with rhodopsin and iodopsin, initiate the nerve impulses, and cause decomposition of the conjugated pigments into protein, retinene, and variable quantities of vitamin A. While one quantum can activate a molecule of rhodopsin and excite a single rod cell, about six cells must be excited to produce an effect which exceeds the threshold. The rhodopsin and iodopsin are resynthesized in the dark; the normal human eye gradually recovers its sensitivity within 30 to 60 minutes. Regeneration of the pigments is inhibited by lack of oxygen. Vitamin A is thus a precursor and a product of the visual cycle. The pigmented retinal epithelium stores vitamin A in considerable concentrations.

A large proportion of ingested vitamin A, and of the provitamins, is catabolized in mammals. Rats can tolerate 100,000 units of vitamin A daily, and the injection of 1,000,000 units does not cause toxic symptoms in man. Normal feces contain small fractions of unabsorbed vitamin A and the provitamins; these substances are not excreted in normal urine, but they are found at times in the urine of patients with cancer, infections, or hepatic or renal disease.

Carotenemia

Patients with diabetes mellitus, myxedema, or severe hepatic disease frequently exhibit nyctalopia and high blood carotene concentrations,

owing to defective hepatic conversion of the provitamins to vitamin A. Marked carotenemia causes a yellow discoloration of the skin (xanthosis cutis); the development of this symptom generally requires a high carotene intake. Xanthosis may be differentiated from jaundice by lack of discoloration of the sclera, by extraction of the orange-yellow carotenoids from the serum by shaking with petroleum ether or acetone, and by the Van den Bergh reaction (page 558).

Avitaminosis A; Keratinizing Metaplasia; Nyctalopia

Deficit of vitamin A causes epithelial atrophy, followed by reparative proliferation of the basal cells to form keratinizing epithelium (keratinizing metaplasia). The skin becomes rough and dry, the sweat and sebaceous glands atrophy, and hyperkeratosis of the pilosebaceous follicles produces papular lesions (phrynoderma, keratosis follicularis, pityriasis rubra pilaris). Keratinizing metaplasia also occurs in the epithelia of the cornea, conjunctiva, tear ducts, middle ear, sinuses, upper respiratory tract, salivary glands, pancreas, renal pelvis, ureters, uterus, and vagina, resulting in diarrhea, increased susceptibility to infections, and a high incidence of urinary calculi. In advanced stages of deficiency, ulceration of the cornea and xerophthalmia develop, and the keratomalacia eventually causes blindness. In children, retardation of growth, and dental defects occur. Various lesions of the central nervous system have been observed in vitamin A deficient animals (myelin degeneration of the cord, medullary tracts and peripheral nerves, and constriction of the optic nerve with resultant blindness). Vitamin A deficiency decreases urea clearance and blood and liver esterase activity.

The hepatic store of vitamin A esters is depleted readily by an inadequate intake, although several months may elapse before symptoms of the deficiency become evident. The vitamin A activity of the blood decreases, and values below 60 international units per 100 ml. are considered diagnostic of avitaminosis A. Even mild deficiencies cause photophobia, nyctalopia (nightblindness or hemeralopia), bulbar conjunctivitis, blepharitis, and keratinization and infection of the corneal epithelium. Nyctalopia is the most easily recognized early symptom of vitamin A deficiency; it is always present when the vitamin A activity of the blood is less than 70 units per 100 ml. This nutritional form of nyctalopia is caused by slow rhodopsin regeneration. The rod vision is affected more than the cone vision. Efficiency of dark adaptation in patients can be measured by an adaptometer (biophotometer).

In chronic diarrhea, biliary obstruction, pancreatic disease, sprue, celiac disease, and other steatorrheas, vitamin A deficiency can result from deficient absorption. The vitamin A reserve is readily depleted by improper reducing diets, and by pregnancy, fevers, and hyperthyroidism. Hepatic vitamin A is low in many diseases, and in hepatoma tissue, but it is in-

creased in diabetes mellitus. Extensive parenchymatous liver damage interferes with vitamin A storage, and with the conversion of the provitamins; in such conditions, vitamin A preparations are administered daily.

In clinical vitamin A deficiencies, from 30,000 to 50,000 units of the vitamin are usually administered daily. The dosage necessary for relief of nyctalopia is variable; in diabetes mellitus, hepatic cirrhosis, and thyroid disease the retinal response is poor, partly owing to deficient utilization of the vitamin by the retina. Administration of thyroid can accelerate dark adaptation in mild nyctalopia. At times, corneal inflammations and chorioretinal disturbances respond to vitamin A administration. The treatment of xerophthalmia and keratomalacia is difficult after corneal ulceration appears. Steatorrhea, biliary obstruction, and hepatic cirrhosis may require prolonged vitamin A administration. The vitamin is used prophylactically in infancy, pregnancy, and lactation. The fish liver oil preparations are given orally; solutions of carotene in oil are used for parenteral injection. Vitamin A ointments have been employed for the treatment of acne, dermatitis, eczema, and skin ulcerations and infections, but the absorption of vitamin A through the skin is uncertain.

THE VITAMIN B COMPLEX

The mixture of B vitamins found in liver, yeast, and other foods consists of seven identified nitrogenous compounds (thiamin, or vitamin B₁; riboflavin, or vitamin B₂; pyridoxin, or vitamin B₆; nicotinic acid, or P-P factor; pantothenic acid, or filtrate factor; biotin, or vitamin H; and *p*-aminobenzoic acid, or chromotrichia factor), and a number of less well characterized vitamins. The latter include folic acid, a growth factor for micro-organisms; vitamin B₉, a chick antianemia factor perhaps identical with folic acid; vitamin B₁₂, an essential factor for the nutrition of pigeons; factor W, essential for rat growth; and the U and gizzard factors for chicks. (The prevention of nutritional gizzard lesions by cholic acid is mentioned on page 246.) A number of these vitamins (*p*-aminobenzoic acid, biotin, folic acid, nicotinic acid, pyridoxin, riboflavin, and thiamin) are synthesized by bacteria in the gastro-intestinal tract. The interrelations of B vitamins and Bios factors are outlined on page 665. Choline is a dietary essential for chicks, turkeys, dogs, and rats (pages 234 and 598); it is classified as a component of the B complex by some authors. Glycine, arginine and glycuronic acid (or *d*-arabinose), a group of dietary essentials formerly termed the cartilage factor, are also necessary for the growth of chicks. The glycine deficiency syndrome of chicks includes imperfect feather formation, muscular weakness and underdevelopment, and low muscle creatine content; it can be prevented by administering either glycine or creatine (page 414).

Thiamin (Vitamin B₁)

This vitamin contains a pyrimidine and a thiazole nucleus (Table 103, page 642). It is soluble in water and in 70 per cent alcohol, and is insoluble in fat solvents. Thiamin is destroyed readily by sulfite and by alkali. It is rather stable to oxygen, but can be converted to a yellow pigment, thiochrome (Table 103, page 642), by a number of oxidizing agents. The chemical determination of thiamin is based on oxidation to thiochrome and colorimetric comparison of the yellow-blue fluorescence of this compound with a standard in a photoelectric fluorometer. Rapid biological assay methods utilize the acceleration of mold growth and yeast fermentation, and the abolition of rat bradycardia. Thiamin is destroyed by prolonged moist heat at temperatures above 100° C. There is little destruction up to one hour of boiling, but losses occur through discarding the aqueous extract of foods (cooking liquor). Broiling, roasting, high pressure cooking, and the use of baking powder in baking cause considerable losses of food thiamin.

Thiamin is widely distributed in plant and animal tissues. In the latter, it exists largely as thiamin pyrophosphate (diphosphothiamin or cocarboxylase), but the major portion in plant tissues is not phosphorylated. Important dietary sources of thiamin include yeast, nuts, pork, whole grain cereals, egg yolk, legumes, liver, and the meats and vegetables listed in Table 105, page 648. Milling processes eliminate the thiamin-rich germ and hull of cereals. Because the average American diet is somewhat deficient in thiamin, such foods as flour, bread, and breakfast cereals are being enriched with the vitamin. The thiamin requirement varies with the body weight, the caloric intake, and the proportions of carbohydrate and fat in the diet. High fat or protein diets spare thiamin, while high carbohydrate intake increases the requirement. Approximately 0.5 mg. of thiamin is necessary per 1000 calories of average food mixture ingested. The recommended daily allowances for humans are: infants, 0.5 mg.; children, from 1.0 to 2.5 mg.; normal adults, 1.9 mg.; pregnant women, 2.5 mg. and lactating women, 3.0 mg. (See Table 104, page 646, for equivalent unit requirements.)

Thiamin is absorbed readily from the normal intestine. The total thiamin concentration of the blood of the normal human adult is $7.5 \pm 2 \gamma$ per cent. About 1γ per cent is in combination with protein in plasma and in milk; 0.5γ per cent is found in the cerebrospinal fluid. The major portion of the blood thiamin (7γ per cent) is present in the leukocytes and erythrocytes, as cocarboxylase. Leukocytes contain about ten times as much total thiamin as erythrocytes. The administration of thiamin increases the blood levels of free thiamin and cocarboxylase. The latter increases in polycythemia, Hodgkin's disease, and myeloid leukemia; it decreases in anemia and avitaminosis B₁.

Storage of thiamin is limited, and the tissue depots are depleted rapidly by thiamin-deficient diets. The heart, liver, and kidney have the highest concentration of the vitamin, while intermediate thiamin levels are found in the adrenal glands, brain, spleen, lungs, and muscles; despite their relatively low concentration, the muscles contain about one half of the total thiamin of the body. Tissue thiamin occurs largely in the form of cocarboxylase, which is synthesized by a phosphorylase present in largest quantity in the liver, kidney, and intestinal mucosa. Cocarboxylase can be synthesized from thiamin and inorganic phosphate by yeast and by isolated intestinal mucosa. Adenosine triphosphate accelerates the synthesis of cocarboxylase. The specific functions of thiamin include:

(a) Maintenance of normal appetite, digestion, and gastro-intestinal tonicity

(b) Maintenance of normal nerve function

(c) Maintenance of normal cardiac tonicity

(d) Maintenance of normal testicular tissue

(e) Coenzymic function in oxidative decarboxylation of carbohydrate intermediates and in condensation reactions of pyruvic acid (pages 102 and 324).

The last-named function, exerted by diphosphothiamin, is fundamental to the physiological effects of the vitamin. Addition of the vitamin to thiamin-deficient brain tissue suspended in glucose, lactate, or pyruvate solution stimulates the oxygen consumption (the catatorulin effect). Cocarboxylase is an active hydrogen acceptor; together with dihydrococarboxylase, it constitutes an oxidation-reduction system. Thiamin accelerates the biological synthesis of carbohydrate, acetylcholine, and citric, α -ketoglutaric, oxaloacetic, and succinic acids. The administration of thiamin to animals maintained on a low choline diet stimulates synthesis of fat from carbohydrate, and deposition of fat in the liver (page 220). Thiamin inhibits the actions of choline esterase and histaminase. Pyri-thiamin (the pyridine analogue of thiamin) inhibits growth of micro-organisms and causes symptoms of thiamin deficiency in mice by metabolic competition with the vitamin.

The kidneys contain a phosphatase which actively hydrolyzes cocarboxylase to thiamin and inorganic phosphate, and approximately 85 per cent of the urinary thiamin is in the free state. The average urinary excretion of thiamin by the normal human adult is $150 \pm 50 \gamma$ daily; the quantity excreted varies with the thiamin intake. More than 90 per cent of ingested thiamin is destroyed in the body; this catabolism is accelerated by exercise, high carbohydrate diets and hyperthyroidism. A portion of the S^{35} of administered isotopic thiamin is converted rapidly to inorganic sulfate and excreted in the urine; later, some radioactive neutral sulfur is eliminated. In animals, the toxic dose of intravenously injected thiamin is from 125 to 350 mg. per kg. of body weight, and fifty times this quantity

when the vitamin is administered orally. These massive quantities cause respiratory failure, but have little effect on the blood sugar.

Thiamin Deficiency (Avitaminosis B₁); Beriberi. The chief effects of thiamin deficiency are on the nervous and cardiovascular systems. Predominant symptoms are referable to the painful degenerative process known as peripheral neuritis or polyneuropathy. In human beings, these symptoms include: fatigue, anorexia, weakness of the extremities, muscular cramps, sensory disturbances (pain, paresthesia, and burning sensations in the feet), edema, precordial pain, dyspnea on exertion, tachycardia (at times bradycardia), myocardial insufficiency, cardiac enlargement, and palpitation (beriberi heart). Cardiac failure can occur before visible nerve degeneration, if the deficiency is acute; while in chronic thiamin deficiency, degeneration of the nervous system, particularly demyelination of the peripheral nerves, is encountered. The ophthalmoplegia of Wernicke's syndrome is due to thiamin deficiency and resultant degeneration of periventricular gray matter. Similar encephalopathy is responsible for the Chastek paralysis of foxes resulting from ingestion of fresh water fish, which contain an enzyme that hydrolyzes thiamin. Adrenal hypertrophy, testicular atrophy, and small myocardial necrotic areas occur in thiamin-deficient animals. The earliest clinical symptoms of thiamin deficiency either are gastro-intestinal in character (anorexia and gastric and intestinal atony), at times associated with neurasthenia, or they are bilateral neurological symptoms of the lower extremities (plantar dysesthesia, calf muscle tenderness, absence of ankle jerk). Children show pallor, loss of weight, restlessness, spasticity, and edema. In advanced beriberi, there is marked polyneuritis which results in muscular incoordination, paralysis, and nerve degeneration. The "wet" form of beriberi is frequently accompanied by hypoproteinemia and cardiac insufficiency, but the edema does not depend solely on these factors. The brain tissue of animals afflicted with beriberi shows diminished oxygen consumption and acetylcholine synthesis, and a reduced respiratory quotient. In such animals, the diminished oxidation is accompanied by high blood levels of lactic and pyruvic acids. Accumulation of these acids is increased by exercise. Glycogenolysis is accelerated, and the blood cocarboxylase is low (pages 310 and 324). The blood thiamin is below 3 γ per cent, and urinary citrate excretion is subnormal, in thiamin deficiency. In rats, the brain and liver cocarboxylase decreases from normal levels of 300 and 500 γ per cent to 100 γ per cent.

In pregnancy, a peripheral neuritis from thiamin deficiency can develop as the result of inadequate diet or hyperemesis gravidarum. Alcoholic polyneuritis is also due to avitaminosis B₁. Parenteral administration of from 5 to 10 mg. of thiamin affords rapid relief from these complications. Neuritis, which is benefited by thiamin, occurs at times in malnutrition, cardiac conditions, anemias, leprosy, pellagra, psychiatric

disorders, chronic gastro-intestinal diseases (diarrheas, intestinal resection, fistulae, sprue, achlorhydria), drug intoxications, and infections. Other conditions which respond to thiamin include the ophthalmoplegia of Wernicke's syndrome, ocular dendritic herpes, Landry's acute ascending paralysis, toxic amblyopia, trigeminal neuralgia, and subacute combined degeneration of the cord. The nerve lesions which develop in advanced thiamin deficiency are rather resistant to thiamin therapy, and they may be partly conditioned by deficiency of some other dietary factor. Thiamin often fails to relieve diabetic neuritis. It is useful for stimulating appetite in patients; it partially counteracts weight loss and other effects of hyperthyroidism, and is used in the preoperative preparation of these patients. Thiamin has been employed prophylactically to prevent x-ray sickness. The edema caused by thiamin deficiency is usually relieved promptly by administration of the vitamin. Small divided doses minimize the urinary excretion of the administered thiamin; they are most effective in therapy. The intravenous administration of glucose and saline to thiamin-deficient patients can occasionally cause rapid development of deficiency symptoms, because of the increased thiamin demand created by accelerated carbohydrate utilization. Thiamin is beneficial after insulin shock therapy, and at times in other neurogenic types of shock.

Riboflavin (Vitamin B₂)

Riboflavin, formerly termed lactoflavin, is a yellow pigment which displays a green fluorescence. It is slightly soluble in water, and insoluble in alcohol and in fat solvents. Riboflavin is widely distributed in foods, usually as the phosphoric esters, cytoflavin and flavin-adenine dinucleotide. Relatively large concentrations are found in liver, yeast, kidney, eggs, nuts, sea foods, meat, milk, cheese, and the green leafy vegetables listed in Table 105, page 648. To provide adequate dietary riboflavin, such foods as bread, flour, and breakfast cereals are being enriched with the vitamin. Determination of riboflavin is usually based on biological assay (acid formation by *L. casei*); it may also be determined fluorometrically. Riboflavin can be extracted quantitatively from tissues by digestion with pepsin. Riboflavin is destroyed readily by alkali and by light. Ordinary cooking does not cause any marked loss, but the vitamin is partially extracted by the cooking liquor. Storage leads to gradual loss, while roasting and baking destroy the major fraction of food riboflavin. The recommended daily allowances for humans are: infants, 1 mg.; children, from 2 to 4 mg.; normal adults, 2.9 mg.; pregnant women, 4 mg.; and lactating women, 5 mg. Equivalent unit requirements are given in Table 104, page 646. The commonly employed Sherman-Bourquin unit is based on the growth response of rats.

Riboflavin is readily absorbed and phosphorylated in the intestinal mucosa. It is transported in the blood chiefly as flavin-adenine dinucleo-

tide. Normal human blood contains approximately 45 γ per cent of total riboflavin; 15 and 80 γ per cent of flavin-adenine dinucleotide have been found in the plasma and erythrocytes, respectively. All mammalian tissues can phosphorylate riboflavin; the vitamin is present in tissues largely as flavin-adenine dinucleotide prosthetic radicals of the flavoproteins. Some free riboflavin has been detected in the retina. The flavin-adenine dinucleotide radical can be synthesized by erythrocytes *in vitro*; its concentration in the erythrocytes and the general tissues is increased by ingestion of the vitamin. The highest concentrations occur in the liver and kidneys (approximately 1.7 mg. per cent, in terms of riboflavin). Intermediate concentrations are found in the heart, spleen, retina, and brain, whereas skeletal muscle has a relatively low content. Animals have a limited storage capacity for riboflavin; but the vitamin is conserved by the tissues more effectively than is thiamin. Hence, the deficiency symptoms develop slowly. Specific functions of riboflavin include:

- (a) Maintenance of normal ectodermal tissue
- (b) Maintenance of normal nerve tissue
- (c) Coenzymic function in dehydrogenation (as flavin-adenine dinucleotide and cytoflavin prosthetic radicals of flavoproteins).

The last-named function is fundamental to the physiological effects. The coenzymic role of the flavoproteins in dehydrogenation is discussed on page 102. Administration of isoriboflavin (the 5,6-dimethyl isomer) or of the phenazine analogue inhibits riboflavin action in mice and rats by metabolic competition.

The urine of normal human adults contains approximately 1.0 ± 0.7 mg. riboflavin daily. The excretion depends on the riboflavin intake; approximately 25 per cent of a 200 mg. oral dose is excreted in the urine. In lactating animals, about 20 per cent of ingested riboflavin appears in the milk. Five thousand times the customary therapeutic dose of riboflavin is not toxic.

Ariboflavinosis (Avitaminosis B₂); Cheilosis; Rosacea Keratitis. The symptoms of riboflavin deficiency in rats include dermatitis, areas of alopecia, vascularization of the cornea, and a type of cataract characterized by autolysis of lens fibers and proliferation of epithelium. High fat diets increase the severity of the symptoms and incite myelin degeneration and gliosis of the spinal cord, and partial paralysis of the legs. In dogs, the deficiency causes corneal opacity and vascularization, dermatitis, spasticity, weakness, coma, and degeneration of medullary sheaths of nerves. Human ariboflavinosis results in glossitis, cheilosis (fissures at the angles of the mouth), oily desquamation around the nose, and filiform face lesions (shark skin dermatitis). The erosions at the angles of the mouth are occasionally observed in pellagra, but cheilosis is a more common condition. Human ariboflavinosis is, at times, manifested as visual disturbances, photophobia, lacrimation, bulbar conjunctivitis, corneal opacity, pigmentation of the iris, and vascularization of the sclerocorneal junction (rosacea keratitis

associated with acne rosacea). In riboflavin deficiency, the hepatic concentrations of *d*-amino acid oxidase, xanthine oxidase, and flavin-adenine dinucleotide are low. Exhaustion of hepatic riboflavin is hastened by exercise, and hepatoma tissue has a low riboflavin content.

The administration of 5 mg. riboflavin, daily, assists nicotinic acid therapy of pellagra, especially when cheilosis is present. Cheilosis is also alleviated by pyridoxin, and at times by nicotinic acid and ascorbic acid; it may therefore represent a multiple deficiency. Riboflavin is beneficial in vascularization of the cornea and in the corneal keratitis of syphilis. In certain cases of nyctalopia, both vitamin A and riboflavin are necessary to improve the visual adaptation. Riboflavin is usually administered in from 2 to 5 mg. doses, either orally or parenterally.

Cataract. While riboflavin deficiency produces a specific opacity of the lens in rats, the administration of this vitamin does not alleviate the experimental types of cataract caused by dinitrophenol, naphthalene, or thallium poisoning, tryptophane deficiency, or high galactose diets (page 313). The cause of human senile cataract is unknown. The incidence of cataract is high in several skin diseases, cretinism, diabetes mellitus, hypoparathyroidism, mongolian idiocy, and myotonia atrophica. In cataractous lenses, the crystallins are partly replaced by albuminoid, and the concentrations of ascorbic acid, sulfhydryl compounds and carbonic anhydrase are lowered markedly. Pathological calcification and cholesterol ester deposition follow the degenerative changes.

Pyridoxin (Vitamin B₆)

This pyridine derivative (Table 103, page 642) is soluble in water and alcohol, and insoluble in fat solvents. Pyridoxin can be determined colorimetrically by coupling with 2,6-dichloroquinonechloroimide. It is relatively thermostable; but, in dilute solutions, it is decomposed by light. Roasting, stewing, and smoking cause 20 to 50 per cent losses of pyridoxin in meat. The best dietary sources of pyridoxin are egg yolk, nuts, whole grain cereals, legumes, kidney, muscle, liver, fish, yeast, and molasses. The major portion of food pyridoxin exists in combined form. Cereal and vegetable oils, and animal fats, exhibit considerable pyridoxin-like activity, owing to their content of essential unsaturated fatty acids. In animal tissues and yeast, pyridoxin is transformed to pseudopyridoxin, which is more active for lactic acid bacteria. Pseudopyridoxin consists of the corresponding aldehyde and amine (pyridoxal and pyridoxamine); these substances are interconvertible through transamination.

Pyridoxin is absorbed readily in the intestine; its specific functions include:

- (a) Maintenance of normal skin epithelium
- (b) Maintenance of normal erythropoiesis
- (c) Maintenance of normal nerve function
- (d) Acceleration of unsaturated fatty acid utilization.

The essential fatty acids (linoleic, linolenic, and arachidonic acids, page 221) are sometimes designated collectively as vitamin F, but the terminology has not gained general approval. These fatty acids are necessary for the maximal physiological activity of pyridoxin, but they cannot replace it. Pyridoxin is apparently necessary for the biological synthesis of fat from protein, while pyridoxal phosphate accelerates decarboxylase activity. The vitamin is converted largely to 4-pyridoxic acid (the acid corresponding to pyridoxal), which is fluorescent and is excreted by the kidney. Normal human urine contains less than 50 γ per cent of pyridoxin; only 8 per cent of a 100 mg. oral dose of the vitamin is excreted in the urine within four hours. The pyridoxin of human urine is partially conjugated with glycuronic or sulfuric acid. The toxic oral dose of pyridoxin, which causes convulsive seizures, is in excess of 5 gm. per kg. of body weight.

Pyridoxin Deficiency (Avitaminosis B₆); Acrodynia, etc. Pyridoxin deficiency in rats produces a dermatitis which resembles acrodynia or pink disease of infants; other symptoms include atrophy and hyperkeratosis of the skin, fatty livers, convulsive epileptiform seizures, decreased transamination, and conversion of tryptophane to xanthurenic acid (4,8-dihydroxyquinoline-2-carboxylic acid). Some of these symptoms also occur in pyridoxin-deficient pigs; dogs and swine develop cardiac failure, hemosiderosis, a microcytic hypochromic anemia, and degenerative changes in the myelin sheaths.

The administration of pyridoxin increases the reticulocyte and erythrocyte counts, and the hemoglobin concentration of pyridoxin-deficient dogs; the plasma iron is abnormally high, and iron alone will not cure this type of anemia. The dermatitis can be alleviated either by pyridoxin or by the administration of essential fatty acids. Clinical pyridoxin therapy is in the experimental stage. Intravenous doses of from 10 to 100 mg. of the vitamin have been reported to relieve cheilosis, seborrheic eruptions, the residual nervous symptoms of pellagra, the neuritis of arsenic poisoning, certain nutritional microcytic anemias, some cases of pseudohypertrophic muscular dystrophy, and Sydenham's chorea. The pyridoxin requirement is increased in hyperthyroidism.

Nicotinic Acid (P-P Factor)

This heat-stable pyridine acid is soluble in water and alcohol and insoluble in ether. The corresponding acid amide (nicotinamide) is more soluble than nicotinic acid. The vitamin can be determined colorimetrically by decomposition with cyanogen bromide and addition of aniline or *m*-phenylenediamine to develop a yellow coloration. Nicotinamide, nicotinuric acid, and the salts and esters of nicotinic acid are convertible to nicotinic acid in animals. These compounds all possess anti-pellagra activity, but trigonelline (the betaine of nicotinic acid, Table 68,

page 369) is physiologically inert. Important dietary sources of nicotinic acid include rice, bran, liver, yeast, meat, peanuts, and fish (Table 105, page 649). Flour, bread, and breakfast cereals are being enriched with nicotinic acid. In foods, nicotinic acid exists chiefly as cozymases. (See page 101 for the chemistry of the cozymases.) The recommended daily allowances for humans are: infants, 5 mg.; children, from 10 to 25 mg.; normal adults, 20 mg.; pregnant women, 25 mg.; and lactating women, 30 mg. A large fraction of the human nicotinic acid requirement can be synthesized by intestinal bacteria.

The cozymases are hydrolyzed in the intestinal tract, and the liberated nicotinic acid is absorbed readily under normal circumstances. Human blood contains 0.65 ± 0.15 mg. per cent of total nicotinic acid, 96 per cent of which is present in the erythrocytes, chiefly as cozymases. The total cozymase (or factor V) content of these cells is 6 ± 1.5 mg. per cent, including about 2 mg. per cent cozymase I and 4 mg. per cent cozymase II. The erythrocytes can synthesize cozymases from nicotinic acid, *in vitro*. The cozymase content of whole blood (normally 2.7 ± 0.7 mg. per cent) varies with the nicotinic acid intake. The total nicotinic acid level of blood is not altered appreciably by meals or in anemias. Persistent high values result from polycythemia and from continued nicotinic acid therapy. In leukemic patients the white cells have a low cozymase content. Human cerebrospinal fluid contains about 70 γ per cent of nicotinamide.

A considerable portion of ingested nicotinic acid is rapidly converted to tissue cozymases in animals. Cozymase II preponderates in mammalian tissues; the liver has the highest cozymase concentration (about 120 mg. per cent, equivalent to 20 mg. per cent of nicotinic acid). The kidneys contain 100 mg. per cent, and adrenal glands also have relatively high cozymase concentrations, while intermediate quantities are found in the lens, brain, and muscle (50 mg. per cent in the latter). Embryonic and tumor tissues exhibit low cozymase concentrations. Sulfapyridine apparently inhibits the enzymatic transformation of nicotinic acid to pyridine nucleotides; it interferes with the growth response of *Staph. aureus* to nicotinic acid, and with nicotinic acid therapy of black tongue in dogs. Pyridine-3-sulfonic acid, the sulfonic analogue of nicotinic acid, inhibits bacterial growth by competition with the vitamin. The specific functions of nicotinic acid include:

(a) Maintenance of normal epithelia in the skin, and in the alimentary and genito-urinary tracts

(b) Maintenance of normal nerve function

(c) Coenzymic function in dehydrogenation (as cozymases I and II).

The coenzymic function (page 101) is considered to be fundamental to the physiological effects of nicotinic acid. Administered nicotinic acid stimulates the secretion of gastric juice. About one-half hour after the ingestion of this acid, or its salts, the skin exhibits transient vasodilatation

and increased temperature, accompanied by sensations of itching, tingling, and burning. Nicotinamide does not produce the vasodilatation. Liver slices can methylate nicotinamide, but not nicotinic acid. When rats are fed diets containing 2 per cent nicotinamide, fatty livers result from the exaggerated demand for methyl donors. Nicotinic acid is partly catabolized in animals. Normal human urine contains from 2 to 30 mg. free nicotinic acid, 2 to 8 mg. N'-methylnicotinamide (nicotinamide methochloride), and 30 to 60 mg. trigonelline, daily. The urinary excretion of these metabolites is proportional to the nicotinic acid intake. About 20 per cent of a 500 mg. dose of ingested nicotinic acid is excreted within four hours, chiefly as methylnicotinamide, trigonelline and possibly nicotinuric acid (pages 370 and 439). A much smaller fraction of ingested nicotinamide is excreted, chiefly as trigonelline. When the latter is administered, it is excreted unchanged. Daily administration of 2 gm. nicotinic acid per kg. of body weight is not toxic to rats or dogs.

Nicotinic Acid Deficiency; Pellagra. Deficiency of nicotinic acid causes black tongue in dogs, and pellagra in human beings and pigs. Human pellagra is endemic in tropical and semitropical countries, including the southern portion of the United States, where the disease tends to recur in the spring, owing to deficient nicotinic acid intake during the winter months. Well developed pellagra is characterized by dermatitis, diarrhea, and dementia. The dermatitis shows bilateral symmetry; it is usually limited to areas which are exposed to light. The skin lesions include erythema, swelling, desquamation, and scarring. Mucous membranes of the alimentary and genito-urinary tracts are also involved; glossitis, stomatitis, and enteritis are common symptoms. Achylia gastrica is found in about two thirds of pellagra patients. In advanced pellagra, peripheral neuritis occurs, together with psychotic symptoms (loss of memory, depression, disorientation, hallucinations, delirium, and dementia). The cozymase content of the liver and muscles is markedly subnormal; the cozymase concentrations of the blood, brain, and kidneys are less affected. The liver shows an increased oxygen consumption. At times, the plasma protein level is decreased; and either macrocytic or microcytic anemia may develop. Pellagra is accompanied by increased excretion of urorosein (page 378). Nicotinic acid deficiency can result from improper absorption of the vitamin, as in chronic alcoholism, ulcerative colitis, sprue, amebic dysentery, carcinoma of the intestine, and so forth. The type of pellagra which accompanies chronic alcoholism exhibits only central nervous symptoms (the encephalopathic syndrome, characterized by clouded consciousness, grasping sucking reflexes, and cogwheel rigidity of the extremities). Blood cozymase levels are reported low in diabetic ketosis, leukemia, pneumonia, and x-ray sickness.

The basic therapy in pellagra is the oral administration of from 500 to 1,000 mg. nicotinic acid, nicotinamide or sodium nicotinate, daily (usually in 50 mg. doses). Children are given 100 mg. daily. Nicotinic acid

specifically cures the mucous membrane lesions and the associated Vincent's infection of the mouth. Frequently, it abolishes the acute gastro-intestinal, mental and nervous symptoms, and it usually causes disappearance of the dermatitis, provided the skin is unbroken. Otherwise, the dermatological lesions heal very slowly. The pellagra syndrome often involves a multiple deficiency. Alleviation of the anorexia, and the cardiovascular and peripheral nervous symptoms, may require the administration of thiamin; cheilosis is benefited by riboflavin or pyridoxin; and the residual nervous symptoms and muscular weakness respond to pyridoxin and pantothenic acid. Pyridoxin, iron salts, or blood transfusions relieve the accompanying microcytic type of anemia, while macrocytic anemia is an indication for the parenteral administration of liver extract. Occasionally, ascorbic acid and vitamin A may be required. Although active vitamin therapy controls the acute manifestations of pellagra, it is very important to provide the patients with an adequate diet, including meat, milk, eggs, and fresh vegetables, and to correct the dietary habits.

Nicotinic acid has been reported to be beneficial in Vincent's angina, multiple sclerosis, senile encephalomyelosis, and Ménière's syndrome, and to relieve the toxic gastro-intestinal and nervous reactions resulting from sulfanilamide, sulfapyridine, and x-ray therapy. It cures the nutritional encephalopathy of chronic alcoholism.

Pantothenic Acid (Filtrate Factor)

Pantothenic acid is soluble in water, and insoluble in ether. The molecule consists of β -alanine and a branched chain hydroxy fatty acid (pantoic acid) united in peptide linkage (Table 103, page 643). Certain micro-organisms can synthesize pantothenic acid from β -alanine. *d*-Pantothenic acid is more than one hundred times as potent, physiologically, as the *l*-isomer. The royal jelly of the bee is the richest known source of pantothenic acid. Important dietary sources of the vitamin include liver, yeast, egg yolk, meat, salmon, sweet potatoes, dairy products, brans, molasses, peanuts, and broccoli. Pantothenic acid is quite stable to light, air, and cooking, but it is destroyed by alkali and heat. It has been estimated that man requires about 10 mg. of the vitamin daily. Calcium pantothenate is absorbed readily from the intestine. Normal human blood contains approximately 20 γ per cent pantothenic acid, most of which is in combination with protein. In fasting rabbits, oral administration of glucose lowers the pantothenic acid blood level. The vitamin is found in all tissues; the concentrations are relatively high in the liver and kidneys, and low in the muscles. The pantothenic acid of the tissues is largely combined with protein, from which it is separated by tryptic digestion or autolysis. The specific functions of pantothenic acid are:

- (a) Maintenance of normal nerve tissue
- (b) Maintenance of normal skin epithelium.

It has been reported that pantothenic acid stimulates bacterial oxidation of lactic and pyruvic acids, and that it is necessary for the synthesis of fat from carbohydrate in rats. The sulfonic acid analogue, pantooyltaurine, inhibits the growth of micro-organisms which cannot synthesize pantothenic acid. Additional pantothenic acid can reverse the effect, indicating competitive combination in the cells.

Administered pantothenic acid is destroyed rapidly or excreted. The urine of a normal adult man contains about 3.5 mg. daily. Oral administration of 1 gm. calcium pantothenate per kg. of body weight does not cause toxic symptoms in dogs.

Pantothenic Acid Deficiency. In chicks, deficiency of this vitamin results in dermatitis, fatty livers, myelin degeneration of the cord, and decreased pantothenic acid content of the tissues. The symptoms of pantothenic acid deficiency in rats and mice include dermatitis, alopecia, myelin degeneration of nerves and spinal cord, cardiac and renal damage, and hemorrhagic cortical necrosis and atrophy of the adrenal glands. Pantothenic acid, therefore, acts as the chick antidermatitis and the rat antiadrenal necrosis factor. Deficiency of pantothenic acid in pigs causes alopecia, colitis, and myelin degeneration. In dogs, the effects include fatty liver, gastro-intestinal symptoms, hemorrhagic degeneration of the kidneys, coma, and convulsions. Adrenal cortical extract can maintain life in these animals. The pantothenic acid level of blood has been reported to be low in pellagra, beriberi, and ariboflavinosis of human beings. The requirement for pantothenic acid is increased in hyperthyroidism. Calcium pantothenate has been administered to human pellagra patients to relieve the residual nervous symptoms and muscular weakness.

Chromotrichia (Anti-Gray Hair) and Anti-Alopecia Factors

Achromotrichia, or graying of hair, can result from vitamin deficiency, copper deficiency, or inherited factors. Certain types of nutritional achromotrichia respond to administration of calcium pantothenate or *p*-aminobenzoic acid, and at times to biotin, folic acid, nicotinic acid, or thiamin. However, only 5 per cent of human achromotrichias respond markedly to any vitamin medication. Anti-spectacled eye and anti-alopecia activities, formerly attributed to inositol, are now assigned to biotin, and to pantothenic acid and riboflavin, respectively.

Folic Acid; Vitamin B_c

Folic acid, a growth factor for micro-organisms, has been isolated in crystalline form. It is present in green leaves, grass, liver, spleen, and kidney. The leukopenia and anemia resulting from sulfonamide administration can be relieved by folic acid. A chick antianemia factor, vitamin B₉, has been obtained as an orange-colored crystalline acid, which may be

identical with folic acid. These substances are apparently related to xanthopterin.

p-Aminobenzoic Acid

p-Aminobenzoic acid occurs in appreciable quantities in yeast, liver, and kidney. In yeast it is combined with a number of molecules of glutamic acid to form a polypeptide. It is largely in combined form in animal tissues, and it can be set free by acid or alkali. *p*-Aminobenzoic acid is determined by the diazotization procedure used for sulfonamides. Rat blood contains approximately 2.4 γ per cent of the vitamin. The hepatic *p*-aminobenzoate concentration is raised by administration of the vitamin, and is lowered by sulfonamides. *p*-Aminobenzoic acid serves as a coenzyme for the bacterial synthesis of methionine; it exerts an inhibitory action on the growth of certain *rickettsiae*. It inhibits synthesis of the thyroid hormone, and the activities of phenol oxidases and of sulfonamides. Sulfonamide-fast types of *Staph. aureus* produce more *p*-aminobenzoic acid than do other strains of the organism. Both *p*-aminobenzoic acid and sulfonamides affect melanin formation (page 476). When rats are fed sulfaguanidine or sulfasuxidine for long periods, they exhibit retarded growth, hypertrophy of the thyroid gland, agranulocytosis, bone marrow aplasia, corneal hemorrhages, hypoprothrombinemia, muscular necrosis, and vascular sclerosis. A number of these symptoms are traceable to deficiency of vitamins normally synthesized by intestinal bacteria, namely, *p*-aminobenzoic acid, biotin, folic acid, pyridoxin, and vitamin K. The corneal hemorrhages are ameliorated by administration of *p*-aminobenzoic acid. This vitamin has proved beneficial for the detoxication of arsenical drugs, and in some cases of vitiligo. *p*-Aminobenzoic acid is acetylated readily by mammals; its acetyl derivative is excreted in the urine. The fatal intravenous dose of *p*-aminobenzoic acid for rabbits is 2 gm. per kg. Rats tolerate large doses, while oral administration of more than 1 gm. per kg. is fatal to dogs.

Biotin (Vitamin H, Coenzyme R, Bios II B)

Biotin is a cyclic acid which contains nitrogen and sulfur; its formula is given in Table 103, page 643. It may be considered as a derivative of imidazole and of thiophene. Biotin is stable to heat, alkali, and most acids, but is inactivated by the action of nitrous acid. *d*-Biotin is the active stereoisomer. There are several biotin vitamers (compounds with similar activities). α -Biotin is found in egg yolk, β -biotin in liver, and miotin in tissues and urine. The oxygen analogue of biotin is 25 to 50 per cent as active as *d*-biotin. In most plant and animal tissues, biotin exists in combined form, and acid hydrolysis is required for its complete extraction. Autolyzed liver contains about 390 γ per cent, and cane molasses 220 γ per cent of biotin; kidney, egg yolk, pancreas, heart, and yeast are other

good sources of the vitamin. Intestinal bacteria synthesize biotin in mammals. Tumor tissue and embryonic tissues contain less biotin than normal adult tissue. Biotin has the highest activity per mg. of any member of the vitamin B complex. It is an important growth factor for yeast and certain bacteria, while other bacteria and fungi can synthesize it from the inactive desthiobiotin or from pimelic acid and cystine. Biotin increases fermentation and respiration of yeast, stimulates oxidation of carbohydrate metabolites in tissues; and it cures the dermatitis produced by the ingestion of excess egg white. Raw egg white contains a glycoprotein, avidin or avidalbumin, which combines with and inactivates biotin (but not niotin). Avidin preparations exhibit lysozyme activity, which is increased by biotin. The avidin-biotin complex is not absorbed from the intestine. The antibiotin effect of avidin disappears when it is given parenterally; injected avidin-biotin complex can cure biotin deficiency. Cooking denatures avidin, and renders it incapable of binding biotin. In human beings, high egg white diets cause scaly dermatitis, pallor, and depression. Symptoms of biotin deficiency in rats include dermatitis, alopecia, edema, and spasticity of the extremities. The vitamin is believed to be an anti-spectacled eye factor, and it prevents perosis. It can incite hepatic fatty infiltration in rats on low fat or low inositol diets. Inclusion of biotin in a synthetic diet increases the incidence in rats of hepatomas from the administration of butter yellow. Avidin does not prevent tumor development. Normal human adults excrete 15 to 110 γ of biotin daily in the urine.

Bios, and Other Nutrilites Required by Heterotrophic Micro-organisms and Plants. The term Bios is applied to the mixture of substances extractable from yeast, which stimulate the proliferation of yeast cells. Bios I is *i*-inositol; Bios II A is β -alanine; and Bios II B is biotin. Various micro-organisms require the following growth factors: the Bios fractions, the identified vitamins B enumerated on page 652, adenine, choline, cozymase, glutamine, guanine, hematin (factor X), oleic acid, pimelic acid ($\text{HOOC CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$) pyruvic acid, thymine, and uracil. Growth and root formation in plants are promoted by the identified vitamins B, *i*-inositol, and the auxins mentioned on page 713.

ASCORBIC ACID (VITAMIN C)

The chemistry of *l*-ascorbic acid has been considered on pages 105 and 283. This keturonic acid derivative is soluble in water, and insoluble in fat solvents. Ascorbic acid is very sensitive to oxidation, especially in alkaline solution in the presence of light and oxygen. It is more stable in dilute acid solution, unless traces of copper are present. Vitamin C can be determined quantitatively, in metaphosphoric acid filtrates, by titration with 2,6-dichlorophenol-indophenol. This indicator, which is red in acid solution and blue in alkaline solution, is reduced by ascorbic acid to a

colorless or leuko form. The best dietary sources of the vitamin are the berries and green leafy vegetables listed in Table 105, page 649, citrus fruits and juices, papayas, and tomatoes. Meat and milk contain relatively little ascorbic acid; breast milk has about five times as much ascorbic acid as does cow's milk. The vitamin C content of many foods is highest during the summer season. Ascorbic acid is the most readily destroyed of all known vitamins. Storage of foods (without refrigeration), addition of baking soda, contact with copper containers, blanching, drying, and ordinary cooking processes cause rapid destruction of ascorbic acid. The vitamin is gradually lost from picked fruits, berries, and vegetables, and this loss is markedly accelerated by cutting or shredding. Unless foods and fruit juices are frozen, their ascorbic acid content decreases rather readily in contact with air; the process is somewhat slower in acid fruit juices. Primates and guinea pigs require ascorbic acid in the diet, although they can apparently synthesize adequate quantities of the vitamin during fetal life; other mammals can synthesize sufficient quantities of the vitamin for the maintenance of normal physiological processes. The recommended daily allowances for humans are: infants, 30 mg.; children, from 50 to 100 mg.; normal adults, 75 mg.; pregnant women, 100 mg.; and lactating women, 150 mg. (Table 104, page 646).

Ascorbic acid is readily absorbed in the small intestine; only 5 mg. daily are lost in human feces. The vitamin is transported in the blood; in the postabsorptive state, the ascorbic acid level rises, particularly in the erythrocytes. When the human body is saturated with the vitamin, the plasma and cerebrospinal fluid contain 1.2 ± 0.6 , and leukocytes about 30, mg. per cent of ascorbic acid. Plasma levels below 0.7 mg. per cent indicate unsaturation. Fetal blood contains two or three times as much ascorbic acid as does maternal blood; the blood level falls rapidly after birth, unless ascorbic acid is administered. The vitamin is assimilated rapidly by tissues. The largest concentrations of ascorbic acid are found in the adrenal cortex, corpus luteum, pituitary, brain, pancreas, thymus, testes, ovaries, spleen, liver, and intestine; muscle and adipose tissue have smaller concentrations. The human body contains about 4 gm. of the vitamin; the storage capacity is limited; hence, the vitamin should be ingested daily. In tissues and tissue fluids, ascorbic acid exists largely in the reduced form. Ammonium chloride administration mobilizes ascorbic acid from the tissues, and sodium bicarbonate has a reverse effect. Injection of corticotropic hormone lowers the ascorbic acid of the adrenal gland temporarily. When in a state of saturation, the adult human daily oxidizes approximately 0.8 mg. ascorbic acid per kg. of body weight, but only one half this quantity when the reserve is low. The specific functions of ascorbic acid include:

(a) Maintenance of normal connective tissue, and of the intercellular materials of mesenchymal origin

(b) Metabolic transfer of hydrogen via the oxidation-reduction system, ascorbic acid-dehydroascorbic acid.

The physiological significance of the last named function is not entirely clarified. It is known that the vitamin assists in the normal oxidation of tyrosine (page 429). Both dehydroascorbic and ascorbic acids exhibit antiscorbutic activity. *d*-Isoascorbic acid has only 5 per cent of the antiscorbutic activity of vitamin C. Administration of glucoascorbic acid causes symptoms of vitamin C deficiency in rats.

The urinary excretion of ascorbic acid depends on the intake and the blood level. Owing to active reabsorption of the vitamin by the renal tubules, there is a threshold, equivalent to an ascorbic acid plasma level of approximately 1.4 mg. per cent, above which the urinary excretion of this substance is accelerated. The urinary excretion following a test dose of ascorbic acid is, therefore, an index to the state of saturation. The urine of a normal adult contains from 10 to 40 mg. ascorbic acid daily; a smaller quantity is excreted in the sweat. Intravenous injection of very large quantities of ascorbic acid does not cause toxic symptoms in mice. Other details of ascorbic acid metabolism are given on page 332.

Ascorbic Acid Deficiency (Avitaminosis C); Scurvy

The scorbutic condition is characterized by an inhibition of fibroblastic formation of intercellular substances (collagen fibers and non-epithelial cement substances). All fibrous tissue is involved, but the bones, cartilage, dentin, and vascular endothelium are particularly affected. The capillary walls are weakened, and hemorrhages occur in the skin, mucous membranes, bones, joints, gingiva, and muscles. In adults, petechial hemorrhages occur around the hair follicles. The attachment of the periosteum is weakened, and subperiosteal hemorrhages result. Osteoblastic activity and bone growth are inhibited in infants and children, and osteoporosis develops. Formation of collagen-poor connective tissue at the epiphyseal junctions leads to disunion and traumatic fragmentation. Retarded healing of fractures and wounds, beading of the ribs, and swelling of joints occur. Bone is absorbed in the alveolar processes; the teeth become loose, and the gingiva are swollen, spongy and hemorrhagic. The odontoblasts undergo degeneration and atrophy, with accompanying absorption of dentin and failure of tooth development. Other symptoms of scurvy include degeneration and weakness of muscles, cardiac enlargement, atrophy of the adrenal glands and bone marrow, defective hematopoiesis, and secondary infections. Classical scurvy is found, at times, in artificially fed infants, but subclinical deficiency is much more common. The diagnosis of scurvy is based principally on roentgenological evidence, and low plasma ascorbic acid levels. In well developed scurvy, the plasma ascorbic acid concentration is below 0.15 mg. per cent; values between 0.15 and 0.7 mg. per cent are found in subclinical scurvy. In scorbutic guinea pigs, the liver esterase, muscle cytochrome oxidase, and muscle succinic dehydrogenase activities are decreased and the oxygen con-

sumption of muscle is low, although the respiration of most tissues is apparently increased. These scorbutic animals excrete homogentisic, *p*-hydroxyphenylpyruvic, and *p*-hydroxyphenylacetic acids after the administration of tyrosine.

Scurvy results, at times, from deficient intake of ascorbic acid in infants, peptic ulcer patients receiving the Sippy diet, and pellagrins. Ascorbic acid deficiency is occasionally caused by destruction of the vitamin in the gastro-intestinal tract, or by failure of its absorption, as the result of achlorhydria, diarrhea, celiac disease, sprue, or excessive administration of iron salts. It may also accompany increased catabolism or utilization of ascorbic acid in cancer, hyperthyroidism, infections, leukemia, and pregnancy.

The administration of ascorbic acid affords prompt relief in scurvy. The vitamin is used as an adjunct in the treatment of intraocular hemorrhages, peptic ulcer, Addison's disease, certain infections (diphtheria, pneumonia, chronic osteomyelitis, rheumatic fever, and tuberculosis), pyorrhea, arsenical dermatitis, chronic lead poisoning, and iron-resistant types of microcytic anemia. Ascorbic acid therapy increases the tensile strength of healing wounds, and aids in the relief and prevention of x-ray sickness. It has little therapeutic value in thrombocytopenic purpuras. Ascorbic acid, administered as citrus juice, is used prophylactically during infancy, pregnancy, and lactation. Owing to their acidity, fruit juices are preferred in achlorhydric conditions. When allergy to citrus fruits exists, pure ascorbic acid is given. As much as 1 gm. ascorbic acid may be administered daily in scurvy; the usual dose for subclinical deficiencies is from 50 to 100 mg. The vitamin is usually given orally, but it can be injected as a sodium phosphate-ascorbic acid solution.

VITAMIN D

At least ten steride derivatives are known to possess antirachitic, or vitamin D activity. The chemistry of some of these substances has been considered on page 197. Vitamins of the D series are soluble in fat solvents and insoluble in water. They are quite stable to acid, alkali, oxygen, and ordinary cooking procedures. Certain unidentified active products are formed from ergosterol and cholesterol by the action of nitrites and dehydrating agents, respectively; but the vitamins D of greatest biological interest are the irradiation products of ergosterol and 7-dehydrocholesterol, namely, vitamin D₂ (calciferol) and vitamin D₃, respectively. The less active vitamin D₄, or 22-dihydrocalciferol, is the irradiation product of 22-dihydroergosterol. Vitamin D₁ is a lumisterol-calciferol coordination compound which is no longer of biological interest. The inactive provitamins D are isomerized to the corresponding vitamins by the action of ultraviolet light, both *in vitro* and in the skin. The most efficient activat-

ing wave lengths are from 280 to 305 $m\mu$ for ergosterol, and from 289 to 297 $m\mu$ for 7-dehydrocholesterol. Stages in the conversion of ergosterol to its isomer, calciferol, are given on page 198. The intermediate isomers (lumisterol and tachysterol), and the irradiation products of calciferol (toxisterol and the suprasterols) do not have antirachitic activity.

Calciferol, or vitamin D_2 , is present in irradiated plant foods, irradiated yeast, and viosterol preparations. Irradiated 7-dehydrocholesterol, or vitamin D_3 , occurs naturally in fish liver oils and animal fats, and in irradiated milk. The fish liver oils contain, in addition, a vitamin D of undetermined constitution. Vitamin D potency is determined by biological assay (recalcification of bones in rachitic rats). In mammals, crystalline calciferol and vitamin D_3 have potencies of 40,000 international units per mg.; but in chickens, vitamin D_3 is much more active than either calciferol or 22-dihydrocalciferol.

The naturally occurring vitamins D are products of animal metabolism; the chief food sources are fish, egg yolk, butter, cream, and liver (Table 105, page 649). The vitamin D activity of cow's milk is only 20 units per quart, yet milk is important for vitamin D metabolism. The vitamin is more efficiently utilized by animals when it is administered with milk, owing to the calcium, phosphate, lactose, and lactalbumin present in this food. The vitamin D content of cow's milk is highest during the summer months, when the animals are exposed to sunlight. Fortified "vitamin D" milk contains added vitamin D concentrate; its average activity is 400 units per quart, while the activity of irradiated milk is about 135 units per quart. It is difficult to estimate the daily vitamin D allowance, because ultraviolet irradiation of 7-dehydrocholesterol in the skin provides a variable fraction of the human vitamin D supply, and because the requirement rises with the calcium : phosphate ratio of the diet. At present, 400 units daily is the recommended allowance for infants, children, and normal adults. A considerable fraction of this requirement can be supplied by exposure to sunlight. Premature infants and pregnant or lactating women should receive from 600 to 800 units daily. The intestinal absorption of vitamin D requires the presence of bile salts. When the vitamin is applied in a suitable oily vehicle, it can be absorbed slowly through the skin.

The vitamin D activity of normal human serum is 110 ± 50 international units per 100 ml.; the activity rises after ingestion of the vitamin. The liver is an important storage depot for vitamin D. The storage capacity of mammals and birds is limited, although large doses of the vitamin can be retained for several months. Small quantities of the vitamin traverse the placenta and the mammary gland. Specific functions of vitamin D include:

(a) Facilitation of calcium absorption in the intestine, which in turn

promotes phosphate absorption, and maintenance of normal plasma calcium and phosphate levels

(b) Maintenance of normal osteoblastic activity and calcification

(c) Maintenance of normal odontoblasts.

Vitamin D is essential for the conversion of phosphate esters to bone salt, under the influence of osteoblasts. The metabolic relations of the vitamin to calcium and phosphate have been discussed on pages 587 to 591, and 593 to 598. Vitamin D is active in parathyroidectomized animals. Normally, about 25 per cent of the dietary vitamin D is lost in the feces. Fecal excretion of the vitamin is increased in biliary obstruction. Human urine contains no vitamin D. The biological synthesis and catabolism of vitamin D have been considered on page 245.

Early determinations of vitamin D toxicity were affected by the presence of toxic irradiation products in the preparations studied. One of these products, toxisterol, causes hypercalcemia and metastatic calcification. The parathormone-like action of the reduction product of tachysterol (dihydrotachysterol or A.T. 10), and its oral administration in tetany, have been considered on pages 624 and 628. The toxic dose of pure vitamin D for human adults is believed to be in the vicinity of 20,000 international units per kg. of body weight. Such massive doses can produce symptoms which resemble parathormone intoxication, namely, anorexia, nausea, vomiting, diarrhea, muscular weakness, muscle and joint pains, resorption of bone, hypercalcemia, hyperphosphatemia, increased excretion of calcium and phosphate, polyuria, renal disease, urinary calculi, and metastatic calcification of the kidneys, blood vessel walls, lungs, and stomach.

Avitaminosis D; Rachitis, Osteomalacia

Vitamin D deficiency causes rachitis in infants and children and osteomalacia in adults. These skeletal diseases are discussed on page 627. Rachitis is characterized by deficient absorption of calcium and phosphate, retarded growth, and muscular weakness, and by fragility, softening, and deformation of bones. The growing portions of the skeleton are poorly calcified, osteoblastic development is inhibited, the epiphysial cartilage is widened, and the diaphysial line of calcification becomes irregular. The visible results include enlargement of the wrists, elbows, ankles and knees, delayed closure of the fontanelles, pigeon breast, bowed legs, curvature of the spine, craniotabes, rachitic rosary, osteoporosis, bone fractures, and defective tooth development. The tissue calcium concentrations are low, parathyroid activity is increased, and blood phosphatase activity is elevated. Hypophosphatemia and low concentration of phosphate esters in the erythrocytes are usually present. At times, the serum calcium concentration is subnormal, and tetany or secondary anemia may develop. In adults, severe vitamin D deficiency causes osteomalacia, characterized by soft bones and deformities. This condition

develops most frequently in pregnant women, because of deficient vitamin D intake and isolation from sunlight.

Vitamin D therapy is important in rachitis and osteomalacia, and at times in tetany and cases of slowly healing fractures. It is also employed in anemias of children, and in certain skin diseases. The vitamin is administered prophylactically in infancy, pregnancy and lactation, also in diarrhea, steatorrhea, and biliary obstruction. While vitamin D assists tooth development in children, it has little effect upon the adult denture. The administration of from 1,000 to 1,500 units daily usually arrests rachitis, but from 10,000 to 30,000 units are given during active rickets, and certain refractory cases may require more than 50,000 units daily. Exposure to sunlight assists vitamin D therapy. Since parenteral injection of oily vitamin D solutions is frequently irritating, and may cause local calcification at the site of injection, the preparations are usually given orally. Approximate potencies of common vitamin D preparations, in international units per gm., are as follows: cod liver oil, from 60 to 350; halibut liver oil, from 500 to 1,200; cod liver oil concentrates, from 1,500 to 8,500; percomorph liver oil (diluted with an equal volume of cod liver oil), 8,500; fortified liver oils, from 9,000 to 10,000; viosterol (calciferol in oil), 10,000; drisdol (calciferol in propylene glycol), 10,000. The last named preparation is miscible with water.

α -TOCOPHEROL (VITAMIN E)

Vitamins of the E series are chroman derivatives, whose chemistry has been considered on page 207. α -Tocopherol (5,7,8-trimethyltolcol) is the most active vitamin E, but β -tocopherol (5,8-dimethyltolcol), γ -tocopherol (7,8-dimethyltolcol), and the esters of the tocopherols also exhibit vitamin E activity. The active stereoisomers are the *d* forms. The tocopherols are soluble in oils and fat solvents, and insoluble in water. They are stable to alkali and to ordinary cooking processes, but are oxidized in the presence of air, ferric salts, and light. Tocopherol can be determined colorimetrically by means of dipyrityl and iron salt. Vitamin E is found in plant oils, leafy vegetables, and cereals, and in small quantities in animal tissues. Wheat germ oil has high vitamin E potency.

The tocopherols are readily absorbed in the small intestine, provided bile salts are present. They exert a sparing action on vitamin A and carotene by acting as antioxidants in the gastro-intestinal tract. The tocopherol concentration of normal human serum is 1.1 ± 0.5 mg. per cent in adults, and 0.9 ± 0.2 mg. per cent in children. The plasma level reaches a peak at approximately 6 hours after the oral administration of tocopherol. There is little storage of the vitamin, and only minute quantities traverse the placenta, the epithelium of the choroid plexus, or the mammary glands. The specific functions of vitamin E include:

(a) Maintenance of normal reproduction (normal placental function, normal fertilized ova, and normal testes)

(b) Maintenance of normal muscle tissue (probably through effects on nerves).

Vitamin E has not been proved to be a dietary essential for humans, and there is no evidence that it functions as a hydrogen carrier. Tocopherols are not excreted in the urine. Mammals can tolerate orally several grams of α -tocopherol per kg. of body weight without signs of toxicity.

Tocopherol Deficiency (Avitaminosis E)

Vitamin E deficiency causes unsuccessful implantation in rats and mice. The characteristics of the fetal pathology include hemorrhage into the amniotic cavity, rarefaction of the mesenchyme, subnormal development of the vascular system, necrosis and resorption of the fetus. In male rats, mice, and chickens, vitamin E deficiency produces sterility, owing to degeneration of the germinal testicular epithelium. This condition is irreversible, whereas the reproductive abnormality in the female can be cured by the administration of vitamin E preparations. Eggs from vitamin E deficient hens fail to hatch, and the chick embryos die as the result of hemorrhage and failure of development of the blastodermal vessels. Vitamin E deficiency in chicks causes exudative diathesis and encephalomalacia.

More closely related to human pathology are the severe muscular dystrophy and progressive paralysis produced by vitamin E deficiency in ducks, rats, rabbits, dogs, and guinea pigs. This hyaline degeneration of striated muscles is accompanied by connective tissue infiltration, calcification, and increased deposition of collagen and cholesterol. The sodium and chloride concentrations of the affected muscle increase, while the concentrations of potassium, glycogen, and phosphocreatine decrease. The oxygen consumption of muscle slices is high, and the tissue phosphate turnover is accelerated by vitamin E deficiency. Since nerve section is said to prevent the dystrophic changes, the muscle pathology may be related to primary degenerative changes in the nerve cells of the spinal cord. (See page 462 for a discussion of human muscular dystrophy.)

Vitamin E is administered to humans either as wheat germ oil, or as pure α -tocopherol; oily solutions of the latter can be injected intramuscularly. The value of these preparations in habitual abortion is controversial; they are ineffective in human sterility. α -Tocopherol is apparently beneficial in the treatment of primary fibrositis, and its monoether with inositol is effective in progressive muscular dystrophy.

VITAMIN K

The chemistry of the naphthoquinone derivatives, vitamin K₁ (α -phyloquinone or 2-methyl-3-phytyl-1,4-naphthoquinone) from plants and

vitamin K₂ from bacteria, is outlined on page 207. These yellow, fat-soluble vitamins are quite thermostable, but they are destroyed by alkali and light. Two synthetic derivatives, 2-methyl-1,4-naphthoquinone and 2-methyl-4-amino-1-naphthol hydrochloride (vitamin K₃), have about 3.3 times the activity of vitamin K₁ per mg., and 4.25 times that of vitamin K₂ (Table 104, page 646). Phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone), a pigment formed by the tubercle mycobacterium, has a slight vitamin K activity.

The principal dietary sources of vitamin K are hog liver, green plant tissues (alfalfa, cabbage, kale, spinach, tomatoes), and certain vegetable and cereal oils. The vitamin is formed in putrefying fish meal by the activity of micro-organisms. A dietary supply of vitamin K is not strictly essential for normal mammals, since intestinal bacteria can synthesize the vitamin. Bile salts are necessary for the intestinal absorption of vitamin K₁, vitamin K₂, and 2-methyl-1,4-naphthoquinone; 2-methyl-4-amino-1-naphthol hydrochloride is water soluble. Ingestion of mineral oil tends to decrease absorption of the vitamin. Vitamin K can be stored in limited quantities by animals. It can traverse the placenta, but fetal storage of the vitamin is limited. The specific function of the vitamin is the maintenance of hepatic prothrombin synthesis. The relations of vitamin K to prothrombin metabolism have been discussed on page 65. The oral administration of 0.35 gm. 2-methyl-1,4-naphthoquinone per kg. of body weight causes hemolytic anemia in animals, while 2 gm. vitamin K₁ per kg. does not cause toxicity in mice.

Vitamin K Deficiency; Hypoprothrombinemia

In chickens, dietary deficiency of vitamin K produces severe hemorrhagic disease; but in adult mammals, the intestinal bacteria synthesize sufficient vitamin K to prevent similar manifestations. Newborn infants exhibit temporary vitamin K deficiency during the first seven to ten days of life; that is, before the intestinal flora is established. Only 0.5 per cent of the newborn exhibit hemorrhage traceable to hypoprothrombinemia. The hemorrhagic diathesis which is encountered in obstructive jaundice, bile fistulae, and extensive intestinal diseases such as chronic ulcerative colitis, celiac disease, multiple polypi, and sprue, is caused by deficient absorption of vitamin K. Deficit of the vitamin is indicated by a prolonged clotting time (page 65).

The use of vitamin K preparations in hypoprothrombinemia has been discussed on page 67. These preparations are not effective in hepatectomized animals, or in human beings with pernicious anemia, severe hepatic disease (cirrhosis, carcinoma, and severe hepatitis), or Banti's disease. The hypoprothrombinemia accompanying relapse of pernicious anemia can be relieved by liver extract therapy. When absorption of the vitamin is inadequate, bile salt must be administered with vitamin K.

preparations (other than 2-methyl-4-amino-1-naphthol hydrochloride). The oral prophylactic dose of 2-methyl-1,4-naphthoquinone, and of 2-methyl-4-amino-1-naphthol hydrochloride, for pregnant women is from 1 to 2 mg. daily. Prophylactic treatment of the mother during the last few days of pregnancy (not less than 4 hours before delivery) protects the newborn. As much as 5 mg. are given when the prothrombin clotting time is seriously prolonged. 2-Methyl-1,4-naphthoquinone, in oily solution or as a saline suspension, can be injected intramuscularly; and 2-methyl-4-amino-1-naphthol hydrochloride, in aqueous solution, can be given either intramuscularly or intravenously. Intravenous injection of the latter raises the blood prothrombin level within two hours unless the liver is severely injured.

VITAMIN P (HESPERIDIN CHALCONE)

The designation, vitamin P, is applied to the substances in citrus fruits which exert an antihemorrhagic effect on capillaries. The most active component is hesperidin chalcone (hesperetin- β -rutoside), whose structure is given on page 644. The rutoside radical of the glycoside is *d*-glucose-6- β -rhamnoside. Another glycoside which reduces capillary fragility is rutin (quercetin rutoside), found in tobacco leaves. Hesperidin chalcone and rutin have limited therapeutic value in the treatment of vascular purpuras.

BIBLIOGRAPHY

VITAMINS

General

- American Medical Association. The Vitamins. Chicago, 1939. (Methods.)
American Medical Association. Handbook of Nutrition. Chicago, 1943.
BRIDGES, M. A. Dietetics for the Clinician. Ed. 4. Philadelphia, Lea and Febiger, 1941.
BUTLER, A. M. Nutritional requirements in infancy and childhood. *Am. J. Digest. Dis. & Nutrition*, 64 : 898, 1942.
DRILL, V. A. Interrelations between thyroid function and vitamin metabolism. *Physiol. Rev.*, 23 : 355, 1943.
ERBS, J. H. Nutritive requirements in pregnancy and lactation. *J. A. M. A.*, 121 : 339, 1943.
EVANS, E. A., JR. The Biological Action of the Vitamins. Chicago, Univ. of Chicago Press, 1942.
ROSENBERG, H. R. Chemistry and Physiology of the Vitamins. Ed. 2. New York, Interscience Pub., 1945.
SCHOPFER, W. H. Plants and Vitamins. Waltham, Chronica Botanica, 1943.
SHERMAN, H. C., and LANFORD, C. S. Essentials of Nutrition. Ed. 2. New York, Macmillan, 1943.

- WILLIAMS, R. J. The significance of the vitamin content of tissues. *Vitamins and Hormones*, 1 : 229, 1943.

Vitamin A

(See references to Carotenoids, page 258; and Chemistry of Vision, page 259.)

- HEILBRON, I. M., *et al.* The chemistry and physiology of vitamin A. *Vitamins and Hormones*, 2 : 155, 1944.
- POPPER, H. Distribution of vitamin A in tissue as visualized by fluorescence microscopy. *Physiol. Rev.*, 24 : 205, 1944.
- WITH, T. K. Absorption, Metabolism and Storage of Vitamin A and Carotene. Copenhagen, Munksgaard, 1940.

Vitamin B Complex; Nutrilites for Micro-organisms

- HALL, R. P. Growth factors for protozoa. *Vitamins and Hormones*, 1 : 249, 1943.
- KNIGHT, B. C. J. G. Growth factors in microbiology. *Vitamins and Hormones*, 3 : 105, 1945.
- LEPKOVSKY, S. Components of the vitamin B₂ complex. *Nutrition Abstr. & Rev.*, 11 : 363, 1942.
- NAJJAR, V. A., and BARRETT, R. The synthesis of B vitamins by intestinal bacteria. *Vitamins and Hormones*, 3 : 23, 1945.
- PETERSON, W. H., and PETERSON, M. S. Relation of bacteria to vitamins and other growth factors. *Bact. Rev.*, 9 : 49, 1945.

Thiamin

- WILLIAMS, R. R., and SPIES, T. D. Vitamin B₁ and Its Use in Medicine. New York, Macmillan, 1939.

Riboflavin

- BOOHER, L. E. Chemical aspects of riboflavin. *J. A. M. A.*, 110 : 1105, 1938.
- HOGAN, A. G. Riboflavin, physiology and pathology. *J. A. M. A.*, 110 : 1188, 1938.
- SHERMAN, H. C., and LANFORD, C. S. Riboflavin, dietary sources and requirements. *J. A. M. A.*, 110 : 1278, 1938.

Pantothenic Acid

- WILLIAMS, R. J. The chemistry and biochemistry of pantothenic acid. *Adv. in Enzymol.*, 3 : 253, 1943.

Nicotinic Acid

- ELVEHJEM, C. A. Relation of nicotinic acid to pellagra. *Physiol. Rev.*, 20 : 249, 1940.

p-Aminobenzoic Acid

- ANSBACHER, S. *p*-Aminobenzoic acid — experimental and clinical studies. *Vitamins and Hormones*, 2 : 215, 1944.

Biotin

- HOFMANN, K. The chemistry and biochemistry of biotin. *Adv. in Enzymol.*, 3 : 289, 1943.
MELVILLE, D. B. The chemistry of biotin. *Vitamins and Hormones*, 2 : 29, 1944.

Ascorbic Acid

- BESSEY, O. A. Vitamin C, methods of assay and dietary sources. *J. A. M. A.*, 111 : 1290, 1938.
KING, C. G. The chemistry of vitamin C. *J. A. M. A.*, 111 : 1462, 1938.
KING, C. G. The physiology of vitamin C. *J. A. M. A.*, 111 : 1098, 1938.
REID, M. E. Interrelations of calcium and ascorbic acid to cell surfaces and inter-cellular substances. *Physiol. Rev.*, 23 : 76, 1943.
SMITH, S. L. Human requirements of vitamin C. *J. A. M. A.*, 111 : 1753, 1938.

Vitamin D

(See references to Action of Light, page 259.)

- REED, C. I., *et al.* Vitamin D. Chicago, Univ. of Chicago Press, 1939.

Vitamin E

- MASON, K. E. Physiological action of vitamin E. *Vitamins and Hormones*, 2: 107, 1944.
SMITH, L. I. The chemistry of vitamin E. *Chem. Rev.*, 27 : 287, 1940.

Vitamin K

- ALMQUIST, H. J. Vitamin K. *Physiol. Rev.*, 21 : 194, 1941.
BRINKHOUS, K. M. Plasma prothrombin, vitamin K. *Medicine*, 19 : 329, 1940.
BUTT, H. R., and SNELL, A. M. Vitamin K. Philadelphia, Saunders, 1941.
DAM, H. Vitamin K. *Adv. in Enzymol.*, 2 : 285, 1942.
DOISY, E., *et al.* Vitamin K. *Chem. Rev.*, 28 : 477, 1941.

AVITAMINOSES

General

- ARING, C. D. The use of vitamins in clinical neurology. *Bull. New York Acad. Med.*, 19 : 17, 1943.
Association for Research in Nervous and Mental Disease. The Role of Nutritional Deficiency in Nervous and Mental Disease. Baltimore, Williams and Wilkins, 1943.
BAER, A. J. The vitamins in ophthalmology. *Am. J. Ophth.*, 26 : 286, 1943.
BICKNELL, F., and PRESCOTT, F. The Vitamins in Medicine. Ed. 2. London, Heinemann, 1945.
DAFT, F. S., and SEBRELL, W. H. Sulfonamides and vitamin deficiencies. *Vitamins and Hormones*, 3 : 49, 1945.
EDDY, W. H., and DALLDORF, G. Avitaminoses. Ed. 3. Baltimore, Williams and Wilkins, 1943.

- ELLER, J. J., and DIAZ, L. A. Vitamins in dermatology. *Urol. & Cutan. Rev.*, 47 : 234, 1943.
- GORDON, E. S., and SEVRINGHAUS, E. L. *Vitamin Therapy in General Practice*. Ed. 2. Chicago, Year Book Publishers, 1942.
- JEGHERS, H. Skin changes of nutritional origin. *New England J. Med.*, 228 : 678, 714, 1943.
- JOLLIFFE, N. Neuro-psychiatric manifestations of vitamin deficiencies. *J. Mt. Sinai Hosp.*, 8 : 658, 1942.
- JONES, I. H., *et al.* Vitamins and the eye, ear, nose and throat. *Laryngoscope*, 53 : 767, 1943.
- PELNER, L. Vitamins in gastro-intestinal disease. *Am. J. Digest. Dis. & Nutrition*, 10 : 414, 1943.
- PERLMAN, H. B. Vitamins in otolaryngology. *Ann. Otol., Rhin. & Laryng.*, 53 : 267, 1944.
- RUFFIN, J. M., *et al.* Early vitamin deficiency and laboratory determinations of vitamin levels. *Gastroenterology*, 3 : 340, 1944.
- SIEGEL, A. E. The vitamins and their deficiencies with special reference to childhood. *Am. J. M. Sc.*, 201 : 136, 1941.
- SPIES, T. D. Principles of diet in the treatment of disease. *J. A. M. A.*, 122 : 497, 1943.
- WARKANY, J. Prenatal nutritional deficiency. *Vitamins and Hormones*, 3 : 73, 1945.
- WILBUR, D. L. Principles in the use of vitamins in treatment. *Gastroenterology*, 1 : 179, 1943.
- WOLBACH, S. B., and BESSEY, O. A. Tissue changes in vitamin deficiencies. *Physiol. Rev.*, 22 : 233, 1942.
- YOUMANS, J. B., and PATTON, E. W. *Nutritional Deficiencies; Diagnosis and Treatment*. Ed. 2. Philadelphia, Lippincott, 1943.

Avitaminosis A; Nyctalopia; Carotenemia

- BESSEY, O. A., and WOLBACH, S. B. Vitamin A, physiology and pathology. *J. A. M. A.*, 110 : 2072, 1938.
- JEGHERS, H. Skin changes of nutritional origin. *New England J. Med.*, 228 : 678, 1943.
- JOSEPHS, H. W. Hypervitaminosis A and carotenemia. *Am. J. Dis. Child.*, 67 : 33, 1944.
- SPECTOR, S., *et al.* Absorption, storage and utilization of vitamin A in the presence of disease. *Am. J. Dis. Child.*, 66 : 376, 1943.
- STEFFENS, L. F. Vitamin A and night blindness. *Am. J. M. Sc.*, 198 : 292, 1939.
- YUDKIN, A. M. Vitamin A with special reference to therapy. *Bull. New York Acad. Med.*, 15 : 406, 1939.

Avitaminosis B₁; Beriberi

- LEWY, F. H. Vitamin B deficiency and nervous diseases. *J. Nerve. & Ment. Dis.*, 89 : 1, 174, 1939.
- WILLIAMS, R. R., and SPIES, T. D. *Vitamin B₁ and Its Use in Medicine*. New York, Macmillan, 1939.

Avitaminosis B₂; Ariboflavinosis; Cheilosis; Cataract

(See references to Chemistry of the Eye, page 259.)

- BELLOWS, J. G. Cataract and Anomalies of the Lens. St. Louis, Mosby, 1944.
BUTLER, R. E. Riboflavin deficiency. *Med. Clin. N. Am.*, 27 : 399, 1943.
COPPING, A. M. Riboflavin nutrition in man. *Nutrition Abstr. & Rev.*, 14 : 433, 1945.
SYDENSTRICKER, V. P., *et al.* The ocular manifestations of ariboflavinosis. *J. A. M. A.*, 114 : 2437, 1940.
WAGENER, H. P. Riboflavin and keratitis. *Am. J. M. Sc.*, 201 : 303, 1941.

Nicotinic Acid Deficiency; Pellagra

- HARRIS, S., and HARRIS, S., Jr. Clinical Pellagra. St. Louis, Mosby, 1941.
SYDENSTRICKER, V. P. The present status of nicotinic acid. *Arch. Int. Med.*, 67 : 746, 1941.

Ascorbic Acid Deficiency; Scurvy

- FAULKNER, J. M. Vitamin C deficiency. *New Internat. Clinics*, 3 : 1, 1941.
McINTOSH, R. Infantile Scurvy. In Brennemann's Practice of Pediatrics. Vol. I. Hagerstown, Prior, 1944.
RALLI, E. P., and SHERRY, S. Adult scurvy. *Medicine*, 20 : 252, 1941.

Avitaminosis D(See references to Rachitis and Osteomalacia, page 638;
and Action of Light, page 259.)

- COBLENTZ, W. W. Physical aspects of ultraviolet radiation in vitamin D therapy. *J. A. M. A.*, 111 : 419, 1938.
LUCE-CLAUSEN, E. M. Clinical aspects of ultraviolet therapy. *J. A. M. A.*, 111 : 311, 1938.
PARK, E. A. The use of vitamin D preparations in the prevention and treatment of disease. *J. A. M. A.*, 111 : 1179, 1938.
REED, C. I., *et al.* Vitamin D. Chicago, Univ. of Chicago Press, 1939.

Avitaminosis E

- PAPPENHEIMER, A. N. Muscular disorders associated with deficiency of vitamin E. *Physiol. Rev.*, 23 : 37, 1943.

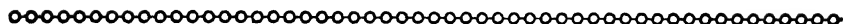
Avitaminosis K

(See references to Blood Clotting, page 126.)

- BRINKHous, K. M. Plasma prothrombin, vitamin K. *Medicine*, 19 : 329, 1940.
BUTT, H. R., and SNELL, A. M. Vitamin K. Philadelphia, Saunders, 1941.
GROSSMAN, A. M. Vitamin K for the pediatrician. *J. Pediat.*, 16 : 239, 1940.
RHODAS, J. E., and FLIEGELMAN, M. T. The use of 2-methyl-1,4-naphthoquinone. *J. A. M. A.*, 114 : 400, 1940.

CHAPTER X

HORMONES AND ENDOCRINOSES



"Aristotle warned us that the parts of the body are not physiologic organs, except in relation to the whole body." — MORRIS R. COHEN

INTRODUCTION: HORMONES AND ANTIHORMONES

The endocrine glands secrete organic substances termed *hormones*, which stimulate, or inhibit, physiological processes in other tissues. The important endocrine glands are: the adrenals, gastro-intestinal mucosae, gonads, pancreas, parathyroids, pituitary, placenta, and thyroid. The nerve endings also liberate neuro-effector hormones (acetylcholine and sympathin), and various embryonic tissues produce inductors, or hormones of differentiation. It has been customary to exclude from the hormone category such tissue products as enzymes (renin), physiologically active catabolites (urea), and the erythrocyte-maturing factor.

The hormones of the pancreas, parathyroids, pituitary, and thyroid are proteins; those of the adrenal medulla and the gastro-intestinal mucosae are protein derivatives; and the characteristic hormones of the adrenal cortex, gonads, and placenta (with the exception of the chorionic gonadotropin) are sterides. Estrogens and thyroglobulin are active after oral administration, while other hormones are largely inactivated in the gastro-intestinal tract or in the liver.

Long-continued parenteral administration of certain hormones causes the gradual development of refractory conditions in which the hormones no longer exert their specific physiological effects. This phenomenon is encountered especially with parathormone and the gonadotropic, lactogenic and thyrotropic pituitary hormones, and with the substances responsible for the metabolic activities of anterior pituitary extracts (page 689). In the refractory conditions, specific inhibitory substances or *antihormones* can be found in the plasma. In many respects, the antihormones resemble immune antibodies; they are not endocrine secretory products. Some hormone antibodies, for example antithyroglobulin, exert relatively little antihormone activity. The antithyrotropic substance displays marked species specificity. Injection of antigonadotropic sera can temporarily produce hypogonad symptoms comparable to those induced by hypophysectomy. In female rats, the gonadotropin content of the pitui-

tary gland is increased by antigonadotropin injection. Insulin is a poor antigen, and its continued administration seldom leads to a refractory insulin-fast condition.

NEUROHYPOPHYSIAL HORMONES (POSTERIOR PITUITARY HORMONES)

Two important hormone fractions, namely, vasopressin or pitressin, and oxytocin or pitocin, are obtainable from the pituicytes of the neural lobe of the hypophysis. *Pituitrin* is the term applied to the posterior pituitary extract which exhibits the activities of both fractions. Studies of fresh posterior pituitary juice, in the ultracentrifuge, indicate that the hormonal activities are attributable to a protein with a molecular weight of approximately 31,000, and an isoelectric point at pH 4.8. It is inactivated by thioglycolic acid or cysteine. The pitressin and pitocin fractions of pituitrin are water-soluble polypeptides of low molecular weight (Table 71, page 397), and isoelectric points at pH 10.8 and 8.5, respectively. They may represent cleavage products of the native hormone. Secretion by the neural lobe is regulated by nerve impulses from the supraoptic and tuberous nuclei of the hypothalamus. Section of the infundibular stalk results in degeneration of the pituicytes, and a marked reduction in the pitressin and pitocin activities of the neural lobe. These hormones are normally secreted into the cerebrospinal fluid; when injected parenterally, the neurohypophysial hormones are destroyed rapidly and are excreted partly in the urine.

Parenteral administration of *pitressin* or *vasopressin* causes constriction of capillaries and arterioles, and brief depression of oxygen consumption and cardiac output due to coronary constriction. The vasoconstriction is not abolished by denervation of blood vessels. The hormone also stimulates contraction of smooth muscle in the gallbladder, intestine, ureter, and urinary bladder; but its most important physiological effect is a marked and rather prolonged antidiuretic action, which results from stimulation of water reabsorption in the renal tubules. Pitressin delays the diuresis which normally follows water ingestion, and it specifically reduces the polyuria of diabetes insipidus. Dehydration causes an increased secretion of pitressin; a nerve reflex is involved, since section of the supraoptico-hypophysial tract inhibits the effect. Large doses of pituitrin, pitressin, or pitocin preparations can cause glycogenolysis, hyperglycemia, increased liver fat, reduced carbohydrate tolerance, achlorhydria, and gastric lesions. Some of these effects may be due to contamination with anterior pituitary hormones (page 689). The gastric lesions do not have any etiological relation to clinical peptic ulcers.

Posterior pituitary hypofunction causes pitressin deficiency and *diabetes insipidus*, which has been discussed on page 619. Experimental diabetes insipidus can be produced by interruption of the nerve tracts between the supraoptic nuclei and the posterior pituitary lobe. The condition is char-

acterized by pronounced polyuria, intense thirst, and loss of weight. Excision of the entire pituitary does not cause polyuria, and the symptoms of diabetes insipidus can be abolished by removal of the anterior lobe. Injection of anterior pituitary extract allows the development of diabetes insipidus in hypophysectomized rats. This indirect action of the anterior lobe has been ascribed to a stimulatory effect of the thyrotropic hormone on thyroid function (page 688). The clinical and experimental forms of diabetes insipidus respond specifically to parenteral or intranasal administration of pitressin or pituitrin preparations. Such extracts are also employed to relieve paralytic ileus and intestinal distension. They have little value in shock and in hypotensive states, since they do not increase blood pressure appreciably in humans.

The *pitocin* or *oxytocin* fraction of posterior pituitary extract stimulates contraction of uterine muscle. This oxytocic response is enhanced by the previous administration of estrogens, and it is antagonized by progesterone. The uterus is most responsive to pitocin at the end of pregnancy, and during parturition. Pitocin preparations are used in obstetrics to control postpartum hemorrhage and to hasten involution of the uterus. However, they tend to cause an undesired elevation of blood pressure in eclamptic patients. Increased secretion of pitocin (as the result of reflexes from pelvic viscera) may be a factor in the normal induction of parturition, but it is not essential, since hypophysectomy does not prevent parturition.

INTERMEDIATE PITUITARY HORMONES

The pars intermedia of the pituitary secretes a melanophoric hormone, *intermedin*, which can disperse pigment granules in the epidermal melanophores and erythrophores of amphibia and fishes. This hormone is related physiologically to the anterior pituitary hormones. In animals which do not have a pars intermedia, intermedin is secreted by the anterior lobe; and the intermedin activity of the gland is not reduced by section of the infundibular stalk. Secretion of the melanophoric hormone is partly controlled through retinal impulses. Seasonal changes in the color and texture of hair and feathers, and the migration and sexual cycles of certain birds and mammals are also traceable to retino-hypophysial activity, for they are inhibited by section of the optic nerve.

The pars intermedia secretes a specific *metabolic factor*, which resembles intermedin in its resistance to pepsin, heat, and dilute acids and alkalis, and in its inactivation by trypsin. Injection of this factor elevates the basal metabolic rate and lowers the respiratory quotient of thyroidectomized or adrenalectomized animals.

ANTERIOR PITUITARY HORMONES

The anterior lobe of the hypophysis has been named the master endocrine organ, because its hormones exert considerable control over the

activities of other endocrine glands (so-called target glands). At present, six specific anterior pituitary fractions are recognized, namely, the growth, follicle-stimulating, luteinizing, lactogenic, thyrotropic, and corticotropic hormones. In addition, extracts of the gland show diabetogenic, glyco-static, glycotropic, and ketogenic activities which have not been allocated to specific fractions. Evidence for the secretion of mammatropic, pancreaticotropic, and parathyrotropic hormones has been obtained in a few species of mammals. The anterior pituitary hormones are proteins which are digested and inactivated by gastro-intestinal enzymes. The usual commercial preparations of the hormones represent impure mixtures.

The acidophile or α cells of the anterior hypophysis are believed to secrete the growth, lactogenic, and thyrotropic hormones, while the corticotropic and gonadotropic substances are ascribed to the basophile or β cells. The chromophobe cells are regarded as being non-secretory; but tumors of these cells can induce certain hypogonadal syndromes, through compression and subsequent degeneration of the β cells (see Fröhlich and Lorain-Levy syndromes, page 686). Experimental extirpation of the anterior hypophysis causes a low basal metabolic rate, subnormal body temperature, slow pulse and respiration, low blood pressure, muscular weakness, hypoglycemia, increased sensitivity to insulin, decreased hepatic and muscle glycogen, abnormal carbohydrate tolerance (increased for oral administration, and decreased for intravenous administration); arrest of growth, development, and lactation; and involution of the adrenal cortex, thyroid, mammary glands, and gonads (and, hence, of accessory sex organs). Partial excision of the gland can produce infantilism, hypogonadism, thyroid enlargement, persistence of the thymus, thickening of the adrenal cortex, inhibition of epiphyseal closure, and some of the metabolic effects noted after total extirpation. Normal animals have four to ten times the necessary quantity of hypophyseal tissue. Not all infantilism is traceable to pituitary deficiency; the non-pituitary types include angioplastic, celiac, pancreatic, rachitic, and renal infantilism, also achondroplasia, cretinism, and microsomia (true dwarfism).

Pituitary tumors can induce either hyperactivity or hypoactivity of the gland. General hypofunction (destruction or atrophy) of the anterior lobe causes *pituitary cachexia*, or Simmonds' disease, which presents the following symptoms: anorexia, gastro-intestinal atony, lowered basal metabolic rate, hypoglycemia, subnormal temperature, hypotension, slow pulse, decreased 17-ketosteroid excretion, amenorrhea, loss of axillary and pubic hair, inhibition of sexual function, anemia, weakness, hypothyroid activity, mental apathy, and coma, and occasionally emaciation and premature senility. The condition is accompanied by splanchnomicria (atrophy of the adrenal cortex, liver, kidneys, ovaries, pancreas, and uterus). Pituitary cachexia can result from postpartum infarction, or from degeneration or necrosis of the anterior hypophysis in chronic infections. The disease is very difficult to differentiate from anorexia nervosa (starvation and loss of

appetite resulting from psychoneurosis), since inanition and severe vitamin deficiency can induce hypopituitarism. The treatment of pituitary cachexia includes parenteral administration of anterior pituitary extracts and gonadotropic preparations, and the oral administration of thyroid.

Growth Hormone (Somatotrophic Hormone)

This substance is probably secreted by α cells of the anterior hypophysis; it is a globulin (molecular weight, 44,250; isoelectric point, pH 6.85), which is inactivated by heat and other denaturing agents. It controls general body growth and exerts a marked influence on skeletal growth through the stimulation of epiphyseal cartilage. The hormone tends to create a positive protein balance, decrease the non-protein nitrogen of blood, and to stimulate hypertrophy of the liver and other tissues. It keeps the epiphyses of growing animals open, but administration of the hormone does not reverse the epiphyseal closure which follows hypophysectomy. Excessive doses of estrogens decrease the secretion of growth hormone and depress growth. Thyroidectomy inhibits growth hormone secretion; injections of growth hormone are effective in thyroidectomized animals but thyroxin improves the growth effect. Repeated administration of growth hormone preparations can extend the growth period, with production of acromegaly or gigantism, and eventual splanchnomegaly and muscular weakness.

Deficiency of the growth hormone during somatic development results in dwarfism and infantilism (also, inhibition of metamorphosis in tadpoles). *Pituitary dwarfism* is a rare clinical condition which shows hereditary tendencies. It is characterized by a small well proportioned body, retarded dentition, wrinkled atrophic skin, and sexual infantilism. At times, the basal metabolic rate is subnormal. Stunted growth, due to deficiency of the hormone, accompanies juvenile Fröhlich, Laurence-Moon-Biedl, and Lorain-Levy syndromes (page 686). The latter represents a type of infantilism which is hereditary. Other conditions believed to involve deficiency of the growth hormone include cretinism and the adult syndrome, *acromicria*, which is characterized by delicate bones, depressed sex function, loss of hair, and cyanosis of the extremities. Growth hormone deficiencies are treated by parenteral replacement, with appropriate anterior pituitary extracts. Administration of extracts which contain the gonadotropic and thyrotropic hormones can induce premature adolescence and hyperthyroidism.

In adults, hyperplasia of the α cells and hypersecretion of the growth hormone causes *acromegaly*, or marked overgrowth of facial bones (mandibles) and bones of the hands and feet. Other symptoms which may be present are enlargement of the tongue and the thyroid and adrenal glands, *splanchnomegaly*, visual changes, pituitary headache, hyperthyroidism, hyperglycemia, glycosuria, decreased carbohydrate tolerance, and in-

creased basal metabolic rate. The enlarging tumor can eventually cause muscular weakness and hypogonadism (amenorrhea, impotence, hot flushes, etc.). Hyperplasia of the α cells during prepuberal growth results in *gigantism*, or increased stature and general skeletal overgrowth. As in acromegaly, late stages of the disease are accompanied by symptoms of β cell hypofunction, but the skeletal changes are not reversed. Hyperpituitary conditions are usually treated by x-rays, or by partial surgical removal of the tumor. Thyroid, androgens, and estrogens are occasionally administered to stimulate epiphyseal union in patients with gigantism.

Gonadotropic Hormones (Gonadotropins)

The β cells of the anterior pituitary lobe produce two gonadotropic glycoproteins, namely, the follicle-stimulating or gametokinetic hormone (FSH), and the luteinizing or interstitial cell-stimulating hormone (LH), which are inactivated by heat, cysteine, and proteinases. Bovine pituitaries contain more luteinizing than follicle-stimulating hormone; the latter predominates in the pituitaries of human beings, rats, sheep, and horses. Piscine, amphibian, avian, and mammalian gonadotropic hormones are not identical. The mammalian gonadotropins apparently contain mannose and glucosamine in 1 : 1 ratio; pig follicle-stimulating and sheep luteinizing hormones contain about 4.5 per cent of each of these sugars, and pig luteinizing hormone about 2.3 per cent of each. The molecular weight of the pig luteinizing hormone is approximately 100,000, and its isoelectric point is pH 7.45; corresponding values for sheep luteinizing hormone are 40,000 and pH 4.6. The secretion of gonadotropic hormones is affected by reflex nervous stimulation and by blood estrogen and androgen levels. The hypophyseal concentration of gonadotropic hormones is increased by the low blood estrogen levels which result from castration or menopause; in these conditions, follicle-stimulating hormone appears in the urine. Normally, the blood and urine of women contain very small quantities of pituitary gonadotropic hormones. Testicular insufficiency increases urinary gonadotropin excretion in males. Continued estrogen or stilbestrol therapy markedly reduces gonadotropic activity, and causes atrophic ovarian changes.

The pituitary gonadotropic hormones tend to act synergistically. Rhythmic variations in their liberation control the menstrual cycles of primates, and the periods of rut or estrus in other mammals. Injection of pituitary gonadotropic preparations causes marked ovarian enlargement, superovulation, and precocious sexual maturity.

In males, the *follicle-stimulating hormone* accelerates spermatogenesis, and development of the testes (growth of seminiferous tubules). In the female, it initiates the first stage of the ovarian cycle by stimulating the maturation of graafian follicles, the secretion of estrogen (estradiol), and the development of ova and granulosa cells. Estrogen secretion is dependent upon the

presence of a little luteinizing hormone, in addition to the follicle-stimulating gonadotropin. When the blood estrogen level is raised sufficiently, the liberation of follicle-stimulating hormone is inhibited, and the liberation of luteinizing hormone is increased. The follicle-stimulating potency of the anterior pituitary is decreased in early pregnancy, and is restored in late stages of pregnancy.

The *luteinizing hormone* stimulates luteinization (*i.e.*, transformation of ovarian theca cells to theca-lutein cells), retention of ova within the luteinized follicles, and inhibition of estrogen secretion. An anterior pituitary factor, termed *luteotropin*, which is probably identical with the lactogenic hormone, maintains the capacity of theca-luteal cells to secrete progesterone. The luteinizing gonadotropin represses the first stage of the ovarian cycle and initiates the second or luteal phase, which produces the uterine effects characteristic of early gestation and pseudopregnancy.¹ The increased blood progesterone level, which results from the luteinizing and luteotropic action, depresses the liberation of pituitary luteinizing hormone. In males, the luteinizing hormone stimulates the interstitial cells of the testes, with production of androgen (testosterone) and development of secondary sex structures. The male pituitary gland tends to secrete luteinizing hormone at a comparatively slow constant rate; injection of androgens stimulates release of this gonadotropin.

Placental Gonadotropic Hormones (Chorionic Gonadotropins). During pregnancy, the placenta, blood, and urine of primates contain a chorionic gonadotropic hormone. This gonadotropin was formerly designated as Prolan B or anterior pituitary-like hormone (APL). It is secreted by the chorionic cells of the primate placenta. The chorionic gonadotropin is a glycoprotein which contains about 18 per cent of acetylglucosamine-digalactose polysaccharide. The molecular weight of the gonadotropin is approximately 100,000, and its isoelectric point is pH 3.3. Injected chorionic gonadotropin exerts luteinizing activity in animals lower than primates. It requires the synergistic action of pituitary follicle-stimulating hormone to produce normal luteinizing effects. In hypophysectomized animals, it causes ovarian enlargement, hypertrophy of interstitial cells, and formation of false corpora lutea. The chorionic gonadotropin has no luteinizing or follicle-stimulating action in female primates; but in males, it stimulates the interstitial cells of the testes, with production of androgen, enlargement of the genitalia, and descent of cryptorchid testes.

Excretion of the chorionic gonadotropin is the basis of the *Aschheim-Zondek test* for pregnancy, in which six injections of urine (total of from 2 to 6 ml.) are administered to an immature female mouse within a period of forty-eight hours. If chorionic gonadotropin is present in the

¹ Pseudopregnancy is induced in certain animals by reflex stimulation of the anterior pituitary gland through sterile mating; it is characterized by development of the corpus luteum, changes in the uterine mucosa, and hypertrophy of the mammary glands.

injected urine, the mouse exhibits (at ninety-six hours) estrus, follicular maturation, luteinization of follicles which have retained ova, and hemorrhages into unruptured follicles. In the commonly used *Friedman modification* of the test, from 10 to 20 ml. of urine are injected, in a single dose, into an adult female rabbit which has been isolated for three weeks or more. Here, the ovarian reaction develops in from sixteen to thirty-six hours. Both procedures detect pregnancy within a few days following the missed menstrual period, since the chorionic gonadotropin appears in the urine very soon after nidation. The excretion of the gonadotropin increases rapidly to a peak at approximately sixty days after the last menstrual period. Following this, the excretion falls to a much lower level, and the substance disappears from the urine a few days after parturition. The serum chorionic gonadotropin concentrations are maximal at approximately the sixtieth day of pregnancy and then decline to slightly lower levels.

A second type of gonadotropic hormone, regarded by some as of placental origin and by subsequent workers as a pituitary hormone, is found in considerable quantities in pregnant mare's serum. It is a glycoprotein with an isoelectric point at pH 2.6. The polysaccharide radical, which constitutes about 26 per cent of the hormone, is a polymer of glucosamine-digalactose. The hormone is inactivated by treatment with cysteine or cyanide. This gonadotropin exhibits the activities of the combined anterior pituitary gonadotropic hormones, and it is active in hypophysectomized animals. Injected pregnant mare serum gonadotropin remains in the circulation longer than other gonadotropins, since it is destroyed slowly and is not excreted in the urine.

Pathology of Gonadotropic Hormones. Hyposecretion of pituitary gonadotropic hormones occurs in pituitary cachexia, pituitary dwarfism, and in the Fröhlich and Laurence-Moon-Biedl syndromes. In juveniles, the *Fröhlich syndrome* (adiposogenital dystrophy) is characterized by obesity, mental deficiency, dwarfism, and subnormal development of the genitalia and secondary sex characteristics. In adults, there are hypogonadal symptoms, disordered menstruation, feminism (in males), obesity, and subnormal temperature and basal metabolic rate. Diabetes insipidus and disturbances of carbohydrate metabolism frequently accompany Fröhlich's syndrome. The *Laurence-Moon-Biedl syndrome* is similar, but it is accompanied by polydactylia and retinitis pigmentosa. Amenorrhea and gonadal hypofunction occur, at times, in late acromegaly and in gigantism from chromophobe adenomas; the sella turcica is usually enlarged, the intracranial pressure is increased, and visual disturbance results from pressure on the optic chiasm. The hypogonadotropic diseases are treated by surgery or x-ray, when they are due to pressure of a tumor, or by parenteral therapy with anterior pituitary extract when primary hypoplasia is responsible for the condition. Thyroid is given if indicated, and reducing diets are employed whenever obesity is pronounced (page 250).

A follicle-stimulating hormone, believed to be of pituitary origin, is excreted in the urine in Cushing's disease (page 689) and at the climacteric. The quantities of estrogen which are usually employed to relieve autonomic disturbances at the climacteric do not inhibit the excessive secretion of follicle-stimulating hormone, but they liberate luteinizing hormone. Cystic ovarian follicles can be produced by excessive administration of pituitary gonadotropins. Chorionic gonadotropin (APL) has been used in the treatment of functional uterine bleeding associated with hypertrophy of the endometrium. Also, in cryptorchidism, from 200 to 500 international units of chorionic gonadotropin are injected on alternate days for from one to two months. The response to this therapy aids in determining the presence of an anatomic barrier and the necessity for surgery. Chorionic gonadotropin has no beneficial effect on ovarian hypofunction.

The excretion of chorionic gonadotropin and its concentration in the placenta and serum are maintained at somewhat higher levels in eclampsia and in the toxemia of pregnancy, and estrogen excretion is low in some instances. (Other features of pregnancy toxemias are discussed on page 460.) There is a tendency toward abnormal chorionic gonadotropin accumulation in the blood of pregnant diabetic women; levels in excess of 200 rat units per 100 ml. of blood after the fifth month are associated with a high incidence of pregnancy toxemia, miscarriage, and stillbirth. Premature diminution in estrogen and progesterone levels may occur, and replacement therapy with estrogen and progesterone in such diabetic patients decreases the infant mortality. The marked excretion of chorionic gonadotropin, which occurs in patients with *hydatiform mole*, *chorionepithelioma*, and the *chorionepithelioma type of testicular teratoma*, is diagnostic for these conditions, and for metastasis of the tumors following hysterectomy.

Lactogenic Hormone (Prolactin)

This hormone is probably formed by the α cells. A crystalline lactogenic protein fraction of anterior pituitary extract has been prepared. The isoelectric point of the lactogenic hormone is at pH 5.7. Sheep and cattle lactogenic hormones have molecular weights of 22,000 and 35,000, respectively. Prolactin is precipitated by cysteine, and is inactivated by pepsin and trypsin, and by acetylation with ketene. It is excreted in human urine. Prolactin is a secretagogue hormone which stimulates lactation following parturition. Hypophysectomy prevents lactation, while injection of prolactin stimulates lactation in normal, hypophysectomized, or ovariectomized mammals, and in males pretreated with estrogen. In pigeons, it stimulates proliferation of the epithelium of the crop glands and the production of caseous crop milk, and it increases the basal metabolic rate in thyroidectomized pigeons. Prolactin incites nesting instincts in birds and fish, and maternal behavior in virgin rats. The luteotropic ac-

tion of the lactogenic hormone is discussed on page 685. Continued injection of prolactin can inhibit the liberation of pituitary gonadotropins and cause atrophy of gonads. Reflex nervous stimulation from suckling stimulates prolactin liberation. When nursing is discontinued, milk production ceases quickly. The quantity of milk depends on prolactin, adrenal cortical hormones, thyroglobulin, and the diet. Thyroidectomy reduces the milk yield, and adrenalectomy inhibits lactation. Administration of cortical extract can maintain lactation in adrenalectomized animals, while desoxycorticosterone is ineffective. During pregnancy, prolactin liberation is inhibited by high levels of estrogens derived from the placenta. Conversely, the secretion of "witches milk" in newborn children may be related to cessation of estrogen influx from the mother. Lactation occurs, at times, in acromegaly and gigantism, because of increased secretion of lactogenic hormone. Women do not respond satisfactorily to parenteral prolactin therapy.

Thyrotropic Hormone (Thyrotropin)

The anterior hypophysis and the thyroid are interrelated through the thyrotropic hormone, which is secreted by the α cells. This hormone is a pseudoglobulin which is inactivated by trypsin, cysteine, and by treatment with ketene. It is present in the blood and urine of normal humans. The thyrotropic hormone can stimulate oxygen consumption of thyroid transplants and tissue cultures, while thyroglobulin reduces it. Injection of the hormone into hypophysectomized animals prevents thyroid atrophy; in normal animals, it causes marked hyperplasia and hypertrophy of the thyroid, accelerated synthesis of thyroglobulin, decrease in the colloid and iodine content of the gland, and such hyperthyroid symptoms as rapid increase in the basal metabolic rate and in the blood iodine concentration. Administration of iodide counteracts the stimulation of the secretory activity, but does not diminish the thyroid hyperplasia. In young animals, hypophysectomy causes thyroid hypoactivity and atrophy, and a lowered basal metabolic rate; it does not decrease the metabolic rate in thyroidectomized animals.

The increased basal metabolic rate in hyperpituitary disease, and the lowered rate in hypopituitary conditions, may be partially related to abnormalities in the liberation of thyrotropic hormone (page 123). Excessive thyroglobulin production (or administration of thyroid or thyroxine) apparently represses the production of thyrotropin and causes thyroid involution; hypothyroidism stimulates liberation of thyrotropic hormone. The thyrotropic activity of the blood and urine is decreased in Simmonds' disease and in hyperthyroidism; it is increased in experimental hypothyroidism and in myxedema. The blood thyrotropin level in thyroid diseases thus varies inversely with the activity of the thyroid gland and its ability to inactivate the hormone. Thiouracil, thiourea, sulfonamides, and related

drugs depress thyroglobulin synthesis (page 707), which in turn leads to release of thyrotropin from the pituitary gland. Repeated injections of a specific thyrotropic hormone preparation cause the development of a refractory state, owing to formation of antithyrotropic substance (page 679). Continued therapy with a single preparation is limited by this effect. In advanced hypothyroidism, atrophy of thyroid tissue precludes thyrotropic activity on the target gland, but the injected hormone can still produce exophthalmus. Thyrotropin apparently induces exophthalmus by causing an increase in the water content of the orbital fat tissue.

Corticotropic Hormone (Adrenotropic Hormone, Corticotropin)

The corticotropic hormone is secreted by the β cells of the anterior pituitary lobe; its molecular weight is 20,000, and its isoelectric point is at pH 4.7. Corticotropin acts directly on the adrenal cortex to maintain the normal structure and function of this gland. Injection of the hormone temporarily lowers the adrenal ascorbic acid and cholesterol concentrations. Hypophysectomy causes marked atrophy of the adrenal cortex, while injection of pituitary extracts can produce hypertrophy and adenomas of the cortex, or it can cause regression of the cortex by inhibiting the liberation of corticotropic hormone. Large doses of desoxycorticosterone may lead to atrophy of the adrenal cortex, perhaps by inhibiting the liberation of corticotropin. The corticotropic hormone has been reported to cause occasional improvement in Addison's disease.

Hyposecretion of the corticotropic hormone occurs in pituitary cachexia; and adrenal hypoplasia also accompanies acrania, anencephalus and hydrocephalus. Corticotropic hypersecretion is encountered in Cushing's disease and acromegaly. *Cushing's disease* is a rather rare condition which is at times associated with pituitary basophilism. The symptoms include sexual dystrophy (amenorrhea and impotence), hypertrichosis of the face and trunk in females and preadolescent males, red or purple liniae atrophiae, hypertension, weakness, acrocyanosis, obesity affecting the trunk, face, and neck; also osteoporosis, hyperglycemia, glycosuria, albuminuria, thyroid and adrenal cortical hypertrophy, and, occasionally, elevation of serum sodium and chloride, and depression of serum potassium. Excessive quantities of corticotropic hormone are excreted in the urine of these patients. Somewhat similar symptoms result from adenomas of the adrenal cortex, but this condition is differentiated by marked excretion of androgen (page 704).

Metabolic Activities of the Anterior Pituitary Lobe

Relations of the anterior hypophysis to carbohydrate metabolism have been discussed on pages 304 to 311. The metabolic activities of anterior

lobe extracts have not been definitely allocated to identified hormone fractions, although the corticotropic hormone evidently participates in some of these activities.

The *ketogenic activity* is a disturbance of hepatic function in fasting animals, which is characterized by hepatic fatty infiltration, ketogenesis, and ketosis. Since the ketogenic effect is reduced by adrenalectomy, the corticotropic hormone may be one of the responsible agents. Animals become refractory to the ketogenic effect when repeated injections of anterior pituitary extracts are given (page 227).

Another temporary effect of anterior pituitary extracts is the *diabetogenic activity*, which is characterized by accelerated hepatic glycogenolysis, decreased carbohydrate tolerance, hyperglycemia, glycosuria, and increased nitrogen excretion. These symptoms appear, at times, in acromegaly; they can be induced experimentally by the injection of anterior pituitary extracts in cats, dogs and rabbits, but not in guinea pigs, mice or rats. The diabetogenic effects disappear after a few days. They are attributable partly to a very heat labile fraction, and partly to the corticotropic hormone; the latter is most intimately related to the gluconeogenesis. In depancreatized animals, hypophysectomy elevates the fasting respiratory quotient, and diminishes the ketosis, gluconeogenesis, hepatic glycogenolysis, hyperglycemia, and glycosuria, but it increases the carbohydrate tolerance only slightly. Removal of the adrenal cortex exerts somewhat similar effects, while thyroidectomy has less influence upon the diabetic symptoms.

Anterior pituitary extracts can exert a *glycotropic* or *anti-insulin* activity in normal, hepatectomized, or hypophysectomized animals; the effect is diminished by adrenalectomy, and it may involve activities of the corticotropic and thyrotropic hormones. The extracts counteract hypoglycemia from injected insulin, but they do not alleviate the hypoglycemia of fasting hypophysectomized or hepatectomized animals. To exert a glycotropic effect, the extracts must be injected several hours before insulin is administered. Hypophysectomy markedly increases the sensitivity of animals to insulin, and the resultant hypoglycemia can be corrected by the injection of adrenal cortex extract. Repeated parenteral administration of increasing doses of crude anterior pituitary extract causes irreversible degeneration of the pancreatic islands of Langerhans in dogs. The resulting type of diabetes mellitus may be regarded as an exhaustion atrophy, analogous to post-hyperthyroid myxedema. The degenerative effect cannot be produced in cats and rats; in fact, rats tend to exhibit an increased pancreatic insulin content following the injections of pituitary extracts.

The *glycstatic activity* of anterior pituitary extracts is the ability to maintain muscle glycogen in hypophysectomized animals; this action is not affected by adrenalectomy.

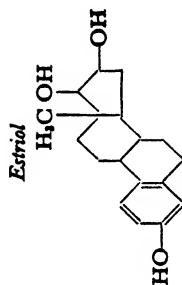
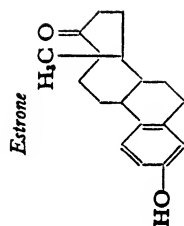
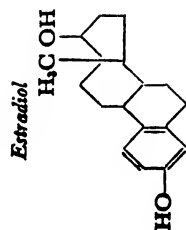
ESTROGENS

The hormones of the ovary are the estrogens and progesterone. The physiologically important estrogens, α and β estradiol, estrone and estriol, are unsaturated fat-soluble derivatives of estrane; their structural relations are shown in Tables 36 (page 194) and 106. The most active estrogen, and the principal estrogenic hormone of the ovary, is the dihydroxy compound, α -estradiol (dihydrotheelin). It is also present in the human placenta and in pregnancy urine. β -Estradiol, obtained from mare's and rabbit's urine, has a different arrangement of radicals at carbon 17; it is less active than α -estradiol, and is not precipitated by digitonin. α -Estradiol is approximately six times as active as its oxidation product, *estrone* or theelin. The international unit of estrogenic activity is equivalent to 0.1 γ of estrone. *Estriol* (theelol) is a trihydroxy derivative which is about one tenth as active as estrone. Its water-soluble glycuronide, known as emmenin, is present in the placenta and in pregnancy urine. The estrogenic activity of this glycuronide is comparatively small. It is effective when given orally, whereas the activity of other natural estrogens is greatly reduced by oral administration. Equilin and equilenin are estrogens of pregnancy mare urine; they have approximately one third and one tenth the estrogenic activity of estrone, respectively. (See Table 36, page 194. The chemistry of the estrogens has been discussed on page 201, and an outline of their metabolic interconversions is given on page 244.)

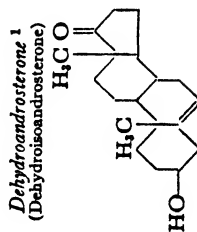
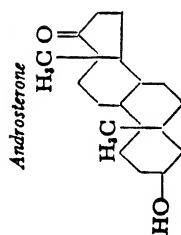
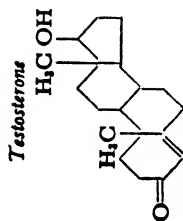
The estrogens are absorbed readily in the intestine, but when ingested, they exert relatively little estrogenic activity, owing to oxidation and conjugation in the liver. Primates convert approximately 90 per cent of administered estrogens to inactive oxidation products. The liver can reversibly oxidize estradiol to estrone and to other products, and it excretes estrone into the bile. Estrone, estradiol, and stilbestrol can be inactivated by laccase. A low protein diet or deficiency of the vitamin B complex inhibits the inactivation of estrogens by rat liver. Transplantation of ovaries to the portal circulation results in a hypogonad condition, and hepatic cirrhosis, at times, induces gynecomastia from an excess of free or unconjugated estrogen. Estradiol is produced by the theca cells of the ovarian follicles, and probably by the interstitial, granulosa, and luteal cells. It has been estimated that the human ovary secretes from 25 to 30 mg. of estradiol per month (at least 10 mg. are required to produce menstruation in an ovariectomized woman). The ovary of the pig contains about 1.5 γ per cent of estradiol and 1 γ per cent of estrone. While the ovary may be regarded as the primary estrogen-producing organ, estrogens can also be formed by the human placenta, testes, and adrenal cortex (as demonstrated in castrates). The human placenta contains about 14 γ per cent of estriol (largely emmenin), 3.5 γ per cent of estrone, and 3.8 γ per cent of estradiol. Estrone is present in the tissues, blood, and urine of

TABLE 106
STERIDE HORMONES

ESTROGENS



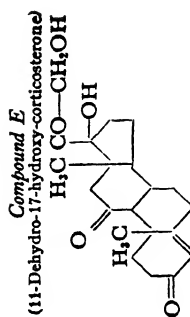
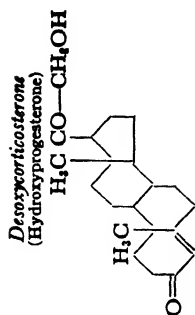
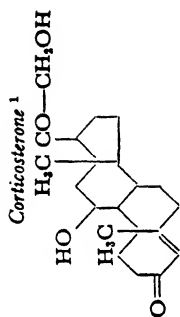
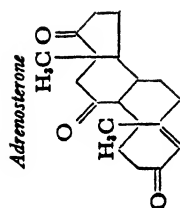
ANDROGENS



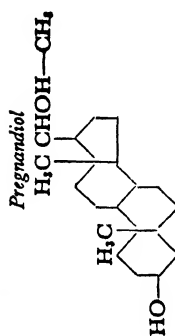
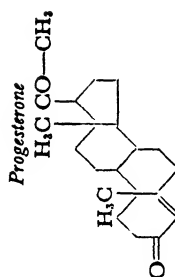
¹ The common designation of this hormone as dehydroisandrosterone is based upon the *trans* relation of its —OH radical to carbon 5.

TABLE 106 (Cont.)

ADRENAL CORTICAL HORMONES



CORPUS LUTEUM HORMONES



¹ In dehydrocorticosterone, a =O radical replaces the —OH at carbon 11; in hydroxycorticosterone, an —OH radical replaces —H at carbon 17.

both males and females. During pregnancy, the estrogenic activity of serum increases gradually from the normal value of approximately 3 international units per 100 ml. to as much as 300 international units at the ninth month. The serum estrogen is apparently combined with plasma protein. The uterus actively converts estrone to estriol when sufficient progesterone is available. The same reaction can also occur in the ovary, and in liver poisoned by cyanide.

The daily urinary estrogen excretion of normal men and women is approximately equal to 100 and 270 international units, respectively. Much less estrogen is excreted by children under ten years of age, or by castrates and eunuchoids; administration of testosterone to eunuchoids increases their estrogen excretion. Boys with progressive muscular dystrophy exhibit a marked urinary excretion of estrogen. The urine of stallions contains much estrone and little androgen; in these animals, estrone excretion is inhibited by castration. Estrone is ordinarily excreted as conjugated ethereal sulfate and glucuronide. Some free estrogen appears in the urine at the mid-interval of the menstrual cycle and immediately before menstruation, when progesterone production is low. The liver conjugates estriol with glucuronic acid to form emmenin, which is a characteristic constituent of the placenta and of pregnancy urine. The urinary estrogen excretion increases markedly after the second month of pregnancy; in the ninth month, the daily output is approximately 300,000 international units (equivalent to approximately 30 mg. of estrone). Estriol glucuronide accounts for 90 per cent or more of this estrogen. Near term, estradiol, estrone, and free estriol appear in the urine; their accumulation in the body sensitizes the uterus to pitocin action. Blood and urine estrogen concentrations are low at times in the toxemias of pregnancy.

The estrogens induce estrus, and stimulate secondary sex organs and sex characteristics in normal or ovariectomized females. They stimulate growth of the uterine muscle and endometrium, hyperemia and motility of the uterus and fallopian tubes, increased capillary permeability in the uterus and vagina, glycogenesis in and thickening of the vaginal epithelium, and lowering of the pH of the vaginal secretion. Prolonged administration of estrogens can produce cystic uterine hyperplasia. This effect is inhibited by simultaneous administration of progesterone or testosterone. The estrogens are necessary for development of the mammary glands; they stimulate growth of the mammary ducts and parenchyma during puberty and pregnancy, and at the onset of estrus. While estrogens aid in the preparation of mammary tissue for prolactin stimulation, they inhibit lactation after it has been established by decreasing the release of lactogenic hormone. They also control hair patterns, and can produce swelling of the nasal mucosa. Uterine hemorrhage and enlargement of the breasts and uterus in the newborn are probably due to sudden arrest of the influx of maternal estrogens. Antenatal estrogenic treatment

of the fetus causes some feminization of male offspring, although such effects are most prominent in developing birds and reptiles.

The estrogens are necessary for the first phase of the menstrual cycle, which includes repair and hypertrophy of the endometrial epithelium, proliferation of the uterine glands, increased motility of the uterus and fallopian tubes, growth of the mammary glands, and thickening, cornification, and glycogenesis in the vaginal mucosa. During this pre-estrus phase, the graafian follicles undergo maturation. Maximal urinary excretion of estrogen (especially estrone) accompanies ovulation at the midpoint of the menstrual cycle, and the excretion (especially estriol) increases again near the close of the second or luteal phase immediately prior to menstruation. Menstruation is initiated by an abrupt decrease in blood estrogen concentration. The endometrial bleeding and necrosis follow vasoconstriction of the sphincters of the coiled arteries, a vascular behavior that can be produced by withdrawal of estrogen, progesterone, or androgen. Prostigmine induces a normal menstrual flow when arrest is due to inadequate vascularity; this action serves as the basis for a pregnancy test.

The parenteral administration of estrogens can stimulate initial enlargement of the pituitary, and liberation of the luteinizing, thyrotropic and corticotropic hormones, with resultant corpus luteum formation, increased thyroid activity, and enlargement of the adrenal cortex. Some estrogens increase the hepatic glycogen of starving animals, and cause retention of sodium and excretion of potassium, as the result of adrenal cortical hyperplasia. Large doses of estrogens inhibit liberation of the growth and follicle-stimulating hormones of the anterior hypophysis; hence, they cause inhibition of follicular development and of spermatogenesis, and eventual atrophy of the gonads. Other effects of excessive estrogen dosage include marked ossification in the medullary cavities of long bones, anemia from contraction of bone marrow, decalcification of pelvic bones, hypercalcemia and lipemia in birds, interruption of lactation and enlargement of the prostate in mammals, and induction of fibromatogenesis and of mammary carcinoma in susceptible laboratory animals. The skeletal effects and the prostatic enlargement can be counteracted by administration of androgens. Injection of estrogens during early pregnancy causes abortion in mammals (other than primates), as the result of endometrial changes.

In adult women, ovariectomy and the atrophic ovarian changes which occur at the *menopause* cause decreased estrogen secretion, atrophy of sex organs, regression of secondary sex characteristics, and a tendency toward obesity. Psychic and vasomotor symptoms (tachycardia, palpitation, dyspnea, hot flushes, nervousness, irritability, insomnia, headache, and fatigue) develop in about 50 per cent of women at the natural menopause. Ovariectomy during the early reproductive period causes severe disturbances, and prevents full development of feminine characteristics.

Amenorrhea (absence of menstrual periods) is physiological prior to the menarche, during pregnancy, and following menopause. It is to be expected after hysterectomy or extensive uterine necrosis from puerperal infection. Primary amenorrhea is caused by hypogonadism, and by hypopituitary inhibition of uterine development. Secondary amenorrhea may result from emotional disturbances, malnutrition, anemia, intoxication, wasting diseases, hyperthyroidism, adrenal cortical tumor, etc. Abnormal bleeding, or *metrorrhagia*, is caused by excessive secretion of estrogen and subnormal progesterone secretion of cystic ovaries, granulosa cell tumors, and other ovarian tumors. In young girls, granulosa cell tumors produce precocious puberty; and after the menopause, they may cause periodic uterine bleeding. *Polymenorrhagia* (shortened menstrual cycle) is usually due to pelvic inflammation, fibromyoma uteri, or uterine retrodisplacement; at times, it accompanies emotional disturbances and hypothyroidism. *Menorrhagia* (excessive menstrual flow) occurs in blood dyscrasias, in myxedema, and in such pelvic inflammatory conditions as adenomyoma, chronic salpingitis, endometritis, fibromyoma uteri, and subinvolution of the uterus.

Estrogens are employed to relieve psychic and vasomotor symptoms, involutional melancholia and osteoporosis at the menopause, to delay hypogonadal effects in ovariectomized women, to suppress painful mammary engorgement and lactation, and to treat pregnancy toxemia and some cases of secondary amenorrhea, dysmenorrhea (painful menstruation), menorrhagia, and metrorrhagia. Estrogen and gonadotropin therapy is, at times, beneficial in amenorrhea; the administration of estrogen causes temporary bleeding, but rarely re-establishes menstrual cycles. Dietary and hygienic measures, and administration of thyroid when the basal metabolic rate is low, are important in the treatment of amenorrhea. Thyroid, chorionic gonadotropin, progesterone, or testosterone propionate occasionally inhibits metrorrhagia and menorrhagia. These conditions may require blood transfusions, administration of liver extract and iron, vitamin B complex therapy, and when other measures fail, curettage, x-ray treatment, or hysterectomy. Estrogen therapy is effective in the management of prostatic carcinoma.

The estrogens, in oily solution, are usually administered by parenteral injection. Benzoic or propionic esters are used when prolonged effects are desired; for example, 2 mg. doses of estradiol benzoate at intervals of 4 days to control symptoms at the menopause. Emmenin, estradiol benzoate and the synthetic estrogenic substance, stilbestrol (diethylstilbestrol or 4,4'-dihydroxy- α,β -diethylstilbene), may be given orally. Stilbestrol has a spacial configuration related to that of the natural estrogens. When injected parenterally, stilbestrol exerts about three times the estrogenic activity of estrone, but it has a smaller mammary-stimulating effect. Like estrone, it releases luteinizing and corticotropic hormones from the pituitary gland. It is destroyed in the liver more slowly than the natural

estrogens; in rabbits it is excreted partly as glucuronide. Other synthetic estrogenic products which may be given orally include benzzestrol, hexestrol, premarin and ethynyl estradiol. Stilbestrol may be slightly more toxic than the natural estrogens; it tends to cause gastro-intestinal disturbances. In large dosage, both the natural and the synthetic estrogens incite fatty degeneration of the liver. The estrogens can be absorbed from the vagina and the skin. Vaginal estrogenic suppositories are used to treat senile vaginitis and kraurosis vulvae, and to induce cornification of immature vaginal epithelium and an acid vaginal secretion in juvenile gonorrheal vaginitis. Oily solutions of estrogens have been applied locally in the therapy of atrophic rhinitis.

PROGESTERONE

This fat soluble hormone of the corpus luteum and the placenta is an unsaturated diketo derivative of pregnane (Tables 36, page 194, and 106). The α and β forms of progesterone are equally active; the international unit of progestational activity is equivalent to 1 mg. of progesterone. Reduction of the unsaturated linkage and the keto radicals of progesterone converts it to the physiologically inert dihydroxy derivatives, pregnandiol (*cis*) and allopregnanediol (*trans*). The chemistry of these substances is considered on pages 201 and 204.

It has been estimated that the human ovary produces from 20 to 25 mg. progesterone per month; a smaller quantity is formed by the adrenal cortex. Administration of deuteriocholesterol to pregnant women leads to the urinary excretion of deuterometabolites of progesterone. Only minute traces of progesterone can be demonstrated in the tissues, blood, and urine of normal or pregnant women, for the hormone is rapidly reduced to pregnandiol in the uterus and liver. In men, desoxycorticosterone can also be converted to pregnandiol. Injected progesterone is not converted quantitatively to pregnandiol glucuronide in human beings. Hysterectomy markedly inhibits pregnandiol production in women; but it does not appreciably affect the conversion of injected progesterone to pregnandiol, which occurs in both sexes. The suggestion has been made that progesterone acts as a hydrogen acceptor in the conversion of estrone to estriol (page 694). The conjugation of pregnandiol with glycuronic acid is a function of the liver. The urinary excretion of sodium pregnandiol glucuronide by the normal female can be diminished by hepatic injury, renal disease, or the absence of a normal endometrium. Women excrete the glucuronide during the luteal phase of the menstrual cycle, commencing within forty-eight hours after ovulation. The excretion attains a maximal daily value of from 4 to 5 mg. about one week prior to menstruation, and it ceases approximately five days later. Marked pregnandiol glucuronide excretion starts at approximately the eightieth day of pregnancy, reaches a maximum of 80 ± 20 mg. pregnandiol per day in the

eighth month, and ceases by the fifth day postpartum. Pregnanediol excretion is lower in cases of pregnancy toxemia which exhibit albuminuria. During the first three months of pregnancy the lutein cells of the corpus luteum are chiefly responsible for progesterone production, whereas in late stages of pregnancy in primates, cats, and guinea pigs, the hormone is formed largely by the placenta. Ovariectomy performed during late pregnancy does not cause abortion in these animals. The urine of the pregnant woman contains traces of sterides chemically related to pregnanediol, namely, the *trans* isomer (allopregnanediol), and the 3-hydroxy, 20-keto derivatives of pregnane and allopregnanediol. The allopregnanediol derivatives are *trans* sterides which are related both to the adrenal cortical hormones and to the androgens (Table 36, page 194). Only traces of pregnanediol are excreted by normal men or by ovariectomized non-pregnant women.

Progesterone is concerned chiefly with the premenstrual or luteal phase of the estrus cycle, which occurs during the two weeks prior to menstruation, and also with pregnancy and pseudopregnancy. Progesterone causes extensive proliferation of the endometrium, and prepares it for implantation of the ovum and nourishment of the embryo. As mentioned previously, estrogen and progesterone are normally secreted in sequence as the result of rhythmic stimulation by the follicle-stimulating, and by the luteinizing and luteotropic hormones, respectively. In the menstrual cycle, the uterus is prestimulated by estrogen, and then sensitized by progesterone to produce decidual tissue in response to implantation of an ovum. Secretory changes occur in the prepared endometrium, its glycogen concentration is raised approximately six times, and the pH of the vaginal fluid increases. Progesterone inhibits follicular maturation, ovulation and estrogen-induced motility of the uterus and tubes. Human ovulation usually occurs near the midpoint of the menstrual cycle. If nidation does not follow, the corpus luteum regresses and is replaced by the corpus albicans, the secretion of progesterone decreases, and the superficial layer of the endometrium undergoes degeneration, necrosis and hemorrhage (menstruation). When pregnancy occurs, the human corpus luteum increases in size until the fourth or fifth month, and it disappears by the seventh month. The progesterone secreted by the corpus luteum and placenta continues to exert its inhibitory effects on ovulation and uterine motility. These actions are necessary for the maintenance of pregnancy, since abortion follows removal of corpora lutea from the rat and other mammals, whose placentae do not produce the hormone. Progesterone also acts synergistically with estrogens to stimulate lobule-alveolar growth of the mammary glands.

Progesterone is administered intramuscularly, in oily solution; 1 to 10 mg. doses are used to prevent threatened or habitual abortion, and to forestall pregnancy toxemias in progesterone-deficient diabetic patients (page 687). The hormone does not stop labor in process or the expulsion

of a dead fetus. Progesterone is used, at times, to relieve menorrhagia, metrorrhagia, dysmenorrhea (painful menstruation), chronic cystic disease of the mammary glands, and the after pains of parturition. Pregnenolone (17-ethynyltestosterone or anhydrohydroxyprogesterone) may be used orally as a substitute for parenteral progesterone therapy.

ANDROGENS

The principal androgens, or male sex hormones, are testosterone, androsterone, dehydroandrosterone (also termed dehydroisoandrosterone), and adrenosterone. Testosterone is an unsaturated ketohydroxy derivative of androstane; androsterone is a reduction product of testosterone, in which the keto and hydroxy radicals have been interchanged; dehydroandrosterone is an unsaturated derivative of androsterone. The structure of these fat soluble sterides is shown in Tables 36, page 194, and 106, page 692. Their chemistry is discussed on page 204. Testosterone is secreted by the testicular interstitial cells of Leydig, under the stimulatory influence of the luteinizing hormone of the anterior pituitary lobe. Adrenosterone is a constituent of the adrenal cortex, while androsterone and dehydroandrosterone are androgenic excretory products. The international androgenic unit is equal to 100 γ of androsterone or 15 γ of testosterone. Esters of testosterone exhibit prolonged androgenic activity. Dehydroandrosterone and adrenosterone are two fifths and one fifth, respectively, as active as androsterone.

Testosterone is found in the testes of fetal mammals, where it is produced in response to maternal gonadotropic stimulation. Only traces are produced during prepuberal life, but at puberty intermittent testicular secretion of testosterone commences in animals which have a rutting season, and continuous secretion begins in primates, rats, and guinea pigs. Orally administered testosterone propionate is absorbed readily in the intestine, but it is only one tenth as effective as the parenterally administered hormone, because of hepatic destruction; methyltestosterone is destroyed more slowly. (See diagram on page 244.) Ingestion of testosterone causes excretion of androsterone, and of smaller quantities of dehydroandrosterone and etiocholanolone (the non-androgenic *cis* isomer of androsterone). The normal daily urinary excretion of such 17-ketosterides (sterones) is approximately 12 ± 6 mg. in men, slightly less in women, and less than 1 mg. in young children. These metabolites are normally excreted in the urine of both sexes as inactive glucuronides, and dehydroandrosterone also as the conjugated sulfate. The average daily androgen excretion is equivalent to approximately 65 international units in men, and 45 international units in women. Smaller quantities of androgens are excreted by children, aged persons, and eunuchoids. 17-Ketosteride and androgen excretion is low in Addison's disease, hypothyroidism, malnutrition, anemia, infections, and liver disease. Ovariectomy and castration

reduce but do not abolish the excretion of androgens, since a considerable fraction of the urinary androgen represents steride metabolites of adrenal cortical hormones. One of the adrenal cortical hormones (compound E) has been transformed into adrenosterone *in vitro*, and considerable quantities of dehydroandrosterone and etiocholanolone (the *cis* isomer of androsterone) are excreted by women afflicted with adrenal cortical tumors and virilism (page 704).

The androgens stimulate the development of secondary sex organs and sex characteristics in mammals, also the growth of the comb, ear lobes, and wattles in capons. Parenteral administration of testosterone can cause atrophy of the endometrium, suppression of menstruation and lactation, and growth stimulation of the mammary parenchyma in women. Administration of sufficiently large quantities of the hormone to pregnant mice, rats or guinea pigs, can cause intersexuality of the female fetus (masculinization and underdevelopment of sex organs). Such free martins develop at times in sheep and cattle, owing to androgen secretion by a male twin fetus. Large doses of testosterone can indirectly inhibit spermatogenesis by decreasing the liberation of pituitary follicle-stimulating hormone; on a weight basis, the androgens cause less gonadotropic inhibition than do the estrogens. Testosterone stimulates renal hypertrophy in mammals (renotropic action), and it increases renal function as measured by inulin clearance and diodrast T_m .

Castration of young males leads to sexual infantilism, enlarged infantile larynx with high pitched voice, failure of masculine type hair growth, retarded epiphysial ossification in the long bones, increased stature, and obesity. In adult males, it can induce vasomotor disturbances, muscular weakness, creatinuria, obesity, mental apathy, regression of secondary sex characteristics, decreased urinary excretion of androgens and estrogens, and increased excretion of follicle-stimulating hormone. *Eunuchoidism*, or subnormal testicular development, results from hypopituitary function in juveniles, or from testicular atrophy induced by cancer, severe malnutrition, mumps, syphilis, typhoid fever, and so forth. The symptoms of eunuchoidism include underdevelopment or regression of secondary sex characteristics, decreased spermatogenesis, subnormal excretion of androgens, infantile type of larynx, soft melanin-deficient skin, fine hair, diminution of sebaceous secretion, poor muscular development, creatinuria, and delayed epiphysial closure. Postpuberal eunuchoidism does not cause lengthening of the bones or infantile regression of the genitalia and the larynx. Chronic alcoholism, fever, and deficiency of thiamin, α -tocopherol, or arginine tend to suppress spermatogenesis, while irradiation of the gonads with x-rays causes permanent sterility. Cryptorchidism is frequently accompanied by degenerative changes in the seminiferous tubules, sterility, and decreased excretion of androgens and estrogens. The relative failure of spermatogenesis in this condition is due to the elevated temperature of the testicular environment. The interstitial testicular

cells do not degenerate entirely, and the male characteristics are lost only partially.

Testicular tumors and adrenal cortical hyperactivity in boys can stimulate androgen excretion and precocious development of secondary sex characteristics (growth of hair, deepening of the voice, enlargement of the penis). The chorionepithelioma type of testicular teratoma is characterized by the excretion of considerable chorionic gonadotropin (page 687). Arrhenoblastomata, and tumors of the adrenal cortex, cause *virilism*, that is, hypertrichosis, striae atrophiae, amenorrhea, and masculinization in women. These patients exhibit reduced estrogen and markedly increased androgen excretion (chiefly dehydroandrosterone and etiocholanolone). The adrenal contains at least two androgenic substances, namely, adrenosterone and 17- β -hydroxyprogesterone. (See also Cushing's disease and adrenogenital syndrome, pages 689 and 704.)

The daily intramuscular injection of from 20 to 25 mg. of testosterone propionate in oil, as replacement therapy in eunuchoidism, is most effective at the normal puberal age. Testosterone causes some masculinization, but the androgens have only a slight stimulatory effect on interstitial testicular cells, and hence the improvement is usually temporary. Large doses of testosterone can induce accelerated bone growth and permanent epiphysial closure. In cryptorchidism, it is less effective than the gonadotropins. Androgens are employed at times in the treatment of menstrual disturbances. Prolonged or excessive administration of testosterone propionate to women is attended by some danger of masculinization and of estrogen deficiency. Testosterone therapy induces positive nitrogen balance in patients with hypopituitarism, Cushing's disease, hyperthyroidism, and other conditions. Testosterone propionate can be absorbed from ointments, and methyltestosterone may be given orally.

ADRENAL CORTICAL HORMONES

These fat-soluble steride hormones are unsaturated derivatives of pregnane (*cis*) and allopregnane (*trans*). Important identified cortical hormones are desoxycorticosterone, corticosterone, dehydrocorticosterone, and dehydrohydroxycorticosterone (compound E). The adrenal cortex also contains a triketo androstane derivative, adrenosterone, which can be formed from compound E by removal of the side chain through the action of alkali or oxidizing agents. (See diagram on page 244.) The structures of these compounds are shown in Tables 36, page 194, and 106, page 693; the chemistry of the cortical hormones has been considered on page 204.

The adrenocortical hormones are readily absorbed in the intestine, but oral administration of the hormones leads to considerable loss of activity. The fatty acid esters exhibit prolonged activity. The formation of these hormones in the adrenal cortex is controlled by the corticotropic hormone of the anterior pituitary. Injection of adrenal cortical extracts into normal

animals produces small increases in blood sugar and hepatic glycogen, and slight retention of sodium and elimination of potassium. Studies in patients and adrenalectomized animals have proved that *corticosterone*, *dehydrocorticosterone*, and compound E, which have an oxygen atom at carbon 11, are concerned with carbohydrate and protein metabolism and with the anti-insulin and other metabolic activities of anterior pituitary extracts (Table 61, page 311, and page 689). These cortical hormones stimulate hepatic gluconeogenesis and glycogenesis, improve muscular efficiency, increase the arginase activity of the liver, minimize the decrease of adrenal cholesterol caused by carbohydrate ingestion, and prolong life in adrenalectomized animals. When injected, compound E induces hyperglycemia and glycosuria in rats, and corticosterone produces marked gluconeogenesis and glycosuria in partially depancreatized or in phlorhizinized adrenalectomized animals. Large doses of the hormones cause atrophy of the adrenal cortex in rats, presumably by depressing the liberation of corticotropic hormone. *Desoxycorticosterone* affects electrolyte distribution in the body. It is necessary for normal absorption of sodium chloride in the intestine, and for the reabsorption of sodium and chloride and the concentration of potassium in the renal tubules. *Desoxycorticosterone*, *corticosterone*, and *dehydrocorticosterone* maintain a normal potassium content in the blood and muscles and normal sodium and chloride concentrations in the extracellular fluid. The cortical hormones having a hydroxyl radical at carbon 17 induce a negative salt balance. Large parenteral doses of *desoxycorticosterone acetate* can depress the serum potassium concentration sufficiently to cause periodic paralysis. (See pages 580 to 586 for further discussion of the relations of *desoxycorticosterone* to mineral metabolism.) *Desoxycorticosterone* (and *progesterone*) resemble *testosterone* in their renotropic action. The unidentified *amorphous fraction* of adrenal cortical extract exerts growth-promoting activity in young adrenalectomized rats, and it assists in the maintenance of renal function. Injected cortical hormones are inactivated readily in mammals. The liver has relatively little action on *desoxycorticosterone*, which is converted partly to *progesterone* and *pregnandiol* in man.

The adrenal cortex is essential to life. Normal individuals have about seven times the minimal necessary mass of adrenal cortical tissue. Adrenalectomy causes inhibition of lactation, loss of appetite, nausea, vomiting, bloody diarrhea, profound muscular weakness, rapid weight loss, subnormal temperature and blood pressure, shock, stupor, and, at times, restlessness or convulsions. Resistance to histamine is decreased, and the adrenalectomized animal is very susceptible to shock. The metabolic abnormalities include low basal metabolic rate, decreased hepatic and renal cytochrome *c* and cytochrome oxidase, lowered hepatic arginase activity, hypoglycemia, and lowered hepatic and muscle glycogen in fasting animals, also decreased sensitivity to insulin, decreased gluconeogenesis and utilization of carbohydrate, retarded absorption of sodium

chloride, low sodium, chloride, and bicarbonate concentrations in the serum, increased muscle potassium, high serum potassium and cholesterol ester concentrations, dehydration, hemoconcentration, decreased tubular reabsorption of sodium, decreased excretion of potassium, diminished respiration and amino acid deamination in the kidney, and renal insufficiency with azotemia and hyperphosphatemia. Adrenalectomized mammals generally die within two weeks.

Deficiency of pantothenic acid causes hemorrhagic necrosis and atrophy of the adrenal cortex in rats and mice; lack of ascorbic acid can also produce adrenal atrophy, while thiamin deficiency causes hypertrophy of the gland. Hemorrhagic necrosis of the adrenal cortex, following acute infectious diseases of childhood, can produce *adrenal apoplexy* or *purpura fulminans*. These patients exhibit marked purpura, hypoglycemia, and azotemia. Chronic adrenocortical insufficiency in humans, known as *Addison's disease*, is characterized by progressive weight loss, fatiguability, asthenia, hypochlorhydria, anorexia, nausea, vomiting, hypotension, melanin pigmentation, and occasional diarrhea or anemia. Excretion of androgens (17-ketosterides) is subnormal in this condition. Acute infections, violent exercise, administration of potassium salts, or withdrawal of sodium from the diet can cause Addisonian crises which are accompanied by shock, dehydration, azotemia, and retention of inorganic phosphate and sulfate. The majority of cases of Addison's disease are caused by tuberculous or syphilitic destruction of adrenal cortical tissue. The untreated disease is usually fatal within a year or two. (See page 622 for further discussion of Addison's disease.)

The treatment of adrenal cortical insufficiency is based upon the administration of desoxycorticosterone acetate, cortical extracts or corticosterone, and a high-carbohydrate diet with extra sodium salt daily. The ingestion of sodium chloride, bicarbonate and citrate greatly reduces the hormone requirement. Readjustment of the mineral intake frequently allows sufficient gluconeogenesis for maintenance, but conditions of stress must be avoided by the patient. Hypoglycemia can develop during treatment with sodium salts and desoxycorticosterone; adequate carbohydrate should, therefore, be provided. Desoxycorticosterone causes rapid re-establishment of normal electrolyte levels and thus improves the circulatory and renal symptoms, but it does not correct the hypoglycemia. Excessive doses of salt and desoxycorticosterone can easily cause edema, peripheral motor paralysis, hypertension, and cardiac insufficiency; hence, the patient should receive adequate thiamin. Desoxycorticosterone acetate overdosage in dogs produces muscular weakness and flaccid paralysis, preventable by administration of potassium chloride, also a syndrome which resembles diabetes insipidus and is associated with high serum sodium levels. The pigmentation of Addison's disease is not specifically relieved by either hormone or ascorbic acid therapy. Desoxycorticosterone acetate exerts only slight activity when given orally; it is usually injected in-

tramuscularly in oily solution, although implantation of pellets of the hormone has been practiced. The water-soluble β -glucoside of desoxycorticosterone is physiologically active. The dosage must be adjusted to the patient's individual requirement, as judged by maintenance of health and body weight. In Addisonian crises, injections of saline, glucose, and cortical hormones are given. Corticosterone is effective in the prophylaxis and treatment of surgical shock. Cortical extract relieves some of the unfavorable symptoms of fever therapy.

Tumors, hyperactivity or dysfunction of the adrenal cortex can cause precocious puberty and adrenal virilism in females. This *adrenogenital syndrome* is characterized by pronounced masculinization and pseudo-hermaphroditism in early life, isosexual precocious maturity and hirsutism in late prepuberal life, and hirsutism and feminization in adult males. Symptoms of adrenal virilism in women include hirsutism, coarse voice, amenorrhea, loss of libido, obesity, diminution in size of breasts, and enlargement of external genitalia. The adrenogenital syndrome is usually accompanied by acne and purple striae, and occasionally by hypertension, osteoporosis, obesity, or diabetes mellitus. The condition is due to a functional derangement of adrenal steride metabolism. Malignant tumors of the adrenal cortex, in either sex, cause marked excretion of androgens. The adrenal cortical hormones can be reduced to pregnane and allo-pregnane derivatives, which are related to progesterone and the androgens, respectively. Desoxycorticosterone has one thirtieth the androgenic activity of androsterone, and one tenth the progestational activity of progesterone. (The latter can prolong survival of adrenalectomized animals.) Adrenosterone, an oxidized cortical hormone, has about one fifth the androgenic activity of androsterone, and the adrenal substance, 17- β -hydroxyprogesterone, is as active as androsterone. It is not surprising that patients with the adrenogenital syndrome excrete abnormally large quantities of dehydroandrosterone and etiocholanolone, and smaller fractions of other androgenic substances of adrenal cortical origin. In males, the adrenogenital syndrome is associated with feminization and increased estrogen excretion; while in females, sodium pregnandiol glycuronide is excreted, even though amenorrhea is present. These symptoms of disordered steride metabolism tend to disappear after surgical removal of adrenal tumors, or following unilateral adrenalectomy in cases of dysfunction.

ADRENAL MEDULLARY HORMONE; ADRENALINE (EPINEPHRINE)

l-Adrenaline is produced by modified sympathetic ganglion cells of the adrenal medulla. The human adrenal gland begins formation of the hormone during the third month of intrauterine life. Adrenaline is a methylated amine derived from tyrosine, probably via tyramine. (See page 376 for the chemistry of this hormone.) The resting adrenal contains about 100 mg. per cent, and normal plasma less than 0.1 γ per cent of

adrenaline. Adrenal production of the hormone is not essential for life, since removal of the adrenal medullae has little effect in animals not subjected to environmental stress. Adrenaline is a sympathomimetic hormone, that is, it reinforces normal sympathetic stimuli under conditions of stress. The secretion of adrenaline is controlled by preganglionic cholinergic fibers, which are active during sympathetic stimulation, as in fear, rage, pain, asphyxia, anesthesia, hemorrhage, strenuous exercise, and exposure to cold.

Adrenaline constricts arterioles in the splanchnic area, the skin, and the mucous membranes; it has little effect on the blood vessels of the lung, and it causes vasodilatation of arterioles in cardiac and skeletal muscle. The hormone causes a rapid temporary increase in systolic blood pressure and in blood volume, accelerates cardiac and respiratory rates, inhibits smooth muscle of the stomach, small intestine, bronchioles and urinary bladder, and stimulates the gallbladder musculature and the sphincters of the gastro-intestinal and urinary tracts. Adrenaline produces elevation of the respiratory quotient and basal metabolic rate, glycogenolysis, hyperglycemia, glycosuria, lacticacidemia, slight lipemia and hypoaminoacidemia, and temporary mobilization of potassium from the liver. The relations of adrenaline to carbohydrate metabolism and clinical glycosuria have been considered in Chapter V, and the effects of adrenaline on the basal metabolic rate, on page 122.

Adrenaline, administered orally, is inactivated rapidly in the gastro-intestinal tract and in the liver. Subcutaneously injected adrenaline disappears from the blood stream within twenty minutes. The intestine, liver, and kidney contain an amine oxidase which rapidly converts adrenaline to methylamine and 3,4-dihydroxyphenylglycolic aldehyde. The inactivation of adrenaline by this enzyme is inhibited by ephedrine and benzedrine. The cytochrome-cytochrome oxidase system and polyphenol oxidase can oxidize adrenaline to an inactive unstable indole derivative, adrenochrome (page 106). This oxidation is inhibited by ascorbic acid and glutathione. A third method of inactivation is hepatic conjugation of adrenaline with sulfate; such conjugates appear in the urine after oral administration of adrenaline. (See page 424 for further details of adrenaline metabolism.)

Hypofunctional disease of the adrenal medulla is unknown. *Paragangliomas* of the adrenal can cause excessive adrenaline secretion, hyperglycemia, and attacks of paroxysmal hypertension; *sympathoblastomas* of the adrenal medulla may induce extensive resorption of bone. These hyperfunctional conditions are treated surgically. Hypertension of adrenal origin can accompany Cushing's disease and the adrenogenital syndrome, but the adrenal medulla is not concerned in ordinary hypertension (page 458).

Adrenaline is applied locally to bleeding surfaces and congested mucous membranes for its vasoconstrictor effect. It is injected with local anesthetic

agents to reduce their rate of absorption, and it is used systemically in anaphylactic shock and in allergic conditions. Adrenaline increases the idioventricular rate in Stokes-Adams disease, and it occasionally resuscitates patients whose hearts have stopped, but it is of little value in surgical shock. The hormone is usually injected subcutaneously; it can be given intramuscularly, as an oily suspension, if prolonged effects are desired. Ephedrine, which inhibits the destruction of adrenaline by amine oxidase, can be given orally; it has a more prolonged effect than does adrenaline.

NEURO-EFFECTOR HORMONES

Sympathin, the substance liberated by adrenergic nerve endings, is closely related to adrenaline. It has been postulated that adrenaline combines with two specific cellular substances to form sympathin E and sympathin I, which mediate sympathetic excitatory and inhibitory effects, respectively (page 424). *Acetylcholine*, the hormone of cholinergic or parasympathetic nerve endings, has been discussed on page 235, and the cholinergic diseases on page 252.

THYROID HORMONE; THYROGLOBULIN

The hormone of the thyroid is a high molecular, iodine-containing globulin; it is produced by the epithelial cells, and is stored as colloid in the alveoli of the gland. Thyroglobulin contains two iodoamino acid units, thyroxine and diiodotyrosine, whose chemistry has been considered on page 376. Experiments with radioactive iodide in thyroidectomized animals show that liver, intestine, and other non-thyroid tissues can form limited quantities of these iodo amino acids. Free diiodotyrosine exhibits only 0.01 per cent of the metabolism-stimulating activity of thyroxine; but as component units of thyroglobulin, the two amino acids exhibit approximately equal activity. For this reason, thyroid preparations can be assayed by their total iodine content. *l*-Thyroxine is the physiologically active stereoisomer. The effects of thyroxine can be inhibited by small quantities of paraxanthine (1,7-dimethylxanthine), which has been found in liver and urine. Thyroglobulin stimulates oxygen usage of tissues *in vitro*, while thyroxine does not.

The normal human thyroid synthesizes daily about 0.33 mg. thyroxine (as thyroglobulin), and the general tissues of the normal adult contain approximately 5 mg. of thyroxine. The formation of thyroglobulin, and the respiration of the thyroid gland, are stimulated by the thyrotropic hormone of the anterior pituitary lobe (page 688). The enlargement of the thyroid which is caused by estrogens, and the diminution in size produced by adrenal cortical hormones or by castration, are believed to be mediated through the thyrotropic hormone. Absence of the thyrotropic hormone leads to atrophic changes in the thyroid, and excessive secretion of thyro-

tropin causes hyperthyroid activity. Simple compensatory hyperactivity and hypertrophy of the thyroid can occur in puberty, pregnancy, and nervous conditions. Dietary deficiency of iodine induces considerable hyperplasia and hypertrophy of the gland without hyperthyroid symptoms. This condition, known as *simple, endemic, or colloid goiter*, has been discussed on page 633. Thiourea ($\text{NH}_2 \cdot \text{CS} \cdot \text{NH}_2$), thiouracil (in which a sulfur atom replaces the oxygen at carbon 2 of uracil), thiocyanate, sulfonamides, and *p*-amino aromatic acids depress thyroid activity and thyroglobulin synthesis (page 418). The thyroglobulin deficiency initiates compensatory release of thyrotropin.

Moderate quantities of thyroid hormone tend to accelerate metabolism in the direction in which it is oriented, namely, anabolism in children and catabolism in the adult. Thyroglobulin is necessary for growth, development, normal intelligence and a normal metabolic rate. The growth-promoting activity of the hormone in young animals is probably dependent on an increased liberation of anterior pituitary growth hormone. The catalytic effect of thyroglobulin and thyroxine on cellular respiration is considered on page 121, and the relations to iodine metabolism on page 610. Daily administration of 0.1 mg. of thyroglobulin raises the basal metabolic rate of a normal man about 10 per cent. The metabolic rate is determined by the balance between thyroid hormone and antithyroid tissue substances, such as paraxanthine. The calorigenic effect of the administered hormone appears in about twenty-four hours, becomes maximal in from eight to ten days, and lasts from five to six weeks. Thyroglobulin causes increased oxidation of all classes of foods, also hepatic glycogenolysis and gluconeogenesis, enhanced hepatic *d*-amino acid oxidase activity, increased unsaturation of muscle phospholipides, decreased fat storage, decalcification of bone, creatinuria, and increased urochrome excretion, creatinine clearance, diodrast T_m , glucose T_m , and renal blood flow. It stimulates gastric motility, intestinal absorption of glucose, erythropoiesis, maturation of the skeleton, epiphyseal union, perspiration, diuresis, and increased sensitivity to adrenaline.

Thyroidectomy causes marked lowering of the basal metabolic rate, and symptoms of cretinism in young animals and of myxedema in adults. Clinical *cretinism* is a type of dwarfism due to congenital thyroid insufficiency during infancy and childhood. An endemic form of the disease occurs in goiter regions. Since thyroxine and the thyrotropic hormone can permeate the human placenta, hypothyroid symptoms do not develop until the infant is from three to five months of age. The symptoms of cretinism include: retarded growth of the skeleton and hair, delayed closure of the fontanelles and epiphyseal ossification in the long bones, sexual underdevelopment, delayed involution of the thymus, coarse features, large tongue, thick dry skin, bradycardia, mental deficiency and, at times, imbecility and deaf mutism. Thyroid deficiency in adults produces *myxedema* which is characterized by the following symptoms:

myxedematous swelling, thick dry skin, sparse hair, bradycardia, cardiac dilatation, increased anterior pituitary activity, liberation of thyrotropin, menorrhagia, muscular weakness, obesity, slow cerebration, and susceptibility to cold. The condition can result from exhaustion atrophy following hyperthyroidism. Both myxedema and cretinism are accompanied by low basal metabolic rate, hypoacidity, decreased blood iodine concentration, diminished glucose absorption, increased carbohydrate tolerance, hypercholesterolemia, lipemia, hemoconcentration, hypovolemia, increased protein and extracellular fluid in the thickened subcutaneous tissue, albuminuria, subnormal urea clearance, decreased excretion of calcium, phosphate, creatine, androgens, and urochrome, and, at times, edema or anemia. Excessive administration of thiocyanate can produce hypothyroidism, associated with exophthalmus and thyroid hyperplasia resulting from increased thyrotropin secretion. Prophylactic doses of iodide prevent this type of goiter. Cretinism results when thiouracil is administered daily to newborn animals. In pregnant animals, thiouracil is transferred to the fetus. The offspring, while normal at birth, soon develop thyroid hyperplasia and hypothyroid symptoms. The effects of thiouracil are prevented by thyroxine administration. (For further discussion of thyroid diseases, see page 632.)

The symptoms of hypothyroidism can be alleviated by oral administration of dried thyroid preparations (which contain 0.2 ± 0.03 per cent of iodine) or of iodized casein. Adequate dosage often causes dramatic improvement within ten days. The replacement therapy is most efficient when it is instituted at an early date (before the age of one year in cretinism) and it must be continued for long periods. It is important to treat hypothyroidism during pregnancy in order to avoid congenital hypothyroidism in the offspring. Thyroxine does not have any advantage over dried thyroid, which is readily absorbed in the intestine. Thyroxine is suitable for intravenous administration, but parenteral medication is seldom important, owing to the latent period in thyroxine and thyroglobulin action. The dosage of thyroid is determined by the severity of the symptoms, the basal metabolic rate, and other criteria. Cumulative effects result because of the slow rate of destruction of thyroid hormone. After the termination of thyroid administration, the basal metabolic rate decreases, but it may not reach the original level for as long as ten weeks. Excessive doses of thyroid can cause nervousness, cardiac pain, palpitation, muscle cramps, or diarrhea. Desiccated thyroid is administered empirically in conditions of low basal metabolic rate other than primary hypothyroidism, as in obesity; it is also used in cutaneous and menstrual disorders, habitual abortion, functional gastro-intestinal conditions, sterility, and certain edemas. Such therapy requires repeated examinations to avoid hyperthyroidism. Administration of thyroid aggravates the symptoms of adrenal cortical insufficiency.

Thyroid hyperactivity, or excessive administration of thyroid, causes

hyperthyroidism or thyrotoxicosis. In *Graves' disease* (Basedow's disease, exophthalmic goiter) the thyroid undergoes diffuse hypertrophy, while in *toxic adenomas* (Plummer's disease) the hyperplasia is local or nodular in type. Exophthalmic goiter can develop rapidly with relatively little enlargement of the thyroid. Only a small percentage of adenomatous goiters become toxic, usually after many years. The symptoms of thyrotoxicosis include: weight loss, increased appetite, anxiety, nervousness, emotional instability, tremor, moist flushed skin, sweating, diarrhea, muscular weakness, myocardial failure, tachycardia, enlargement of the thyroid and heart, and, at times, exophthalmus and amenorrhea. The exophthalmus is probably due to increased thyrotropin secretion. Hyperthyroidism is accompanied by increased synthesis of thyroglobulin, high basal metabolic rate, elevation of blood iodine levels (although thyroglobulin itself does not appear in the circulation), increased excretion of iodine, increased rate of glucose absorption, decreased carbohydrate tolerance, increased hepatic glycogenolysis and gluconeogenesis, hyperglycemia, glycosuria, lipopenia, hypocholesterolemia, hypochlorhydria, low blood thyrotropin level resulting from rapid inactivation of the hormone by the hyperactive thyroid gland, increased parathyroid activity, hypervolemia, polycythemia, osteoporosis, increased excretion of calcium, phosphate and urochrome, creatinuria, negative nitrogen balance, and increased catabolism of vitamin A, thiamin, and ascorbic acid. The thyroid crisis, which frequently occurs after partial thyroidectomy without adequate preoperative preparation, is characterized by extreme nervousness, vomiting, diarrhea, fever, delirium, marked cardiac symptoms, and coma. (See further discussion of hyperthyroidism on page 633.)

The most effective methods of treatment of hyperthyroidism are thiouracil therapy and subtotal resection of the gland. Important preoperative preparation in Graves' disease includes rest, a high calorie, high carbohydrate diet, and administration of sedatives, thiamin, ascorbic acid, calcium salt, thiouracil, and iodide. After the operation, glucose and saline are given parenterally, and the preoperative treatment is continued in order to prevent or minimize thyroid crisis. Thiouracil is at present the most effective substance for the medical control of hyperthyroidism. Daily oral administration of 0.1 to 1 gm. of thiouracil depresses the synthesis of thyroglobulin, lowers the basal metabolic rate, and relieves other symptoms of the condition. Thiouracil (or combined thiouracil and iodide) therapy is therefore used for preoperative management. At least 6 mg. of iodine are administered daily, in the form of saturated potassium iodide solution or Lugol's solution (10 per cent potassium iodide and 5 per cent iodine). The iodide usually decreases the basal metabolic rate, and causes remission of the characteristic symptoms of thyrotoxicosis within twenty-four hours; the symptoms return when iodine medication is stopped. This effect of iodide has diagnostic value in suspected hyperthyroidism associated with only moderate increase in the basal metabolic rate. Iodide

therapy is effective for approximately three weeks, after which a refractory state may develop and continued administration of iodide can initiate re-appearance of serious thyrotoxic symptoms. The temporary remission is due to storage of the hormone in the colloid, and inhibition of thyroglobulin liberation. After the refractory state has developed, renewed storage cannot be effected for some time. Smaller doses of iodide (approximately 2 mg. iodine per week) in the form of Lugol's solution (compound solution of iodine) or of iodized table salt repress the hyperplasia and hypertrophy of simple goiter. The gland usually involutes toward a normal state when iodide administration is begun prior to exhaustion atrophy.

PARATHYROID HORMONE (PARATHORMONE)

This hormone is secreted by the chief cells of the parathyroid gland, under the stimulus of subnormal plasma calcium ion concentration. It is a protease, which is rapidly inactivated by the gastro-intestinal proteinases. Parenteral administration of parathormone causes decalcification of bone, hypercalcemia, elevated serum citrate, hypophosphatemia, increased serum phosphatase activity, increased erythrocytic phosphate ester concentration, diuresis, and negative calcium and phosphate balances. The relations of the hormone to calcium and phosphate metabolism have been discussed on pages 588 to 596.

Complete parathyroidectomy causes death within a few days, unless a high calcium intake is provided. Hypofunction of the parathyroid gland, or *tetany*, is characterized by paresthesia in the extremities, tachycardia, vascular spasm, increased neuromuscular excitability, muscular twitching, spasmophilia, and in severe cases by convulsions, asphyxia and respiratory failure. The effects are due to the hypocalcemia, which is accompanied by hyperphosphatemia and decreased excretion of calcium and phosphate. In chronic tetany, ectodermal disturbances such as cataract, loss of hair, brittleness of nails, and enamel defects, appear. The acute effects are peripheral in nature; the calcium content of the brain, muscle and cerebrospinal fluid is not appreciably lowered in tetany. Latent tetany can be detected by Chvostek's sign (twitching following tapping over the facial nerve), Trousseau's sign (accoucheur's hand induced by obstruction of circulation), and Erb's sign (increased excitability of muscle to galvanic stimulation). Tetany often appears temporarily after partial thyroidectomy. The tetany which accompanies rachitis, osteomalacia and celiac disease is caused by deficient absorption of calcium. (See page 623 for further details of hypoparathyroidism.)

The official solution of parathormone, which has an activity of 100 units per ml., can be administered subcutaneously for relief of tetany. The effects of the injected hormone appear in from three to four hours, are maximal at eighteen hours, and continue for approximately thirty-six hours. Clinical use of the hormone is controlled by repeated estimation of serum

calcium and inorganic phosphate levels, and of urinary calcium. Because of the latent period in parathormone action, acute tetany is alleviated more rapidly by intravenous injection of a calcium salt and by oral administration of vitamin D, milk and acidifying diuretics. A specific parathormone preparation is effective only temporarily, owing to the development of the refractory state. (See antihormones, page 679.) Hence, the blood calcium ion concentration is best maintained by a high calcium, low phosphate diet and by oral administration of dihydrotachysterol (0.5 per cent solution in oil), aluminum hydroxide, and calcium salts. The milk intake is reduced in infantile tetany, because the calcium of milk is utilized less effectively than the phosphate. Vitamin D and calcium salts are used for the tetany associated with rachitis, osteomalacia, sprue, and celiac disease; acidifying salts and calcium therapy are most efficient in gastric tetany. Parathormone has been used in the treatment of lead poisoning, nephrotic edema, and pulmonary hemorrhage.

Repeated injections of parathormone cause acute toxic hyperparathyroidism. In this condition, the serum calcium concentration rises to a peak, and then declines as the refractory state develops. The symptoms of parathormone intoxication include lethargy, muscular weakness, vomiting, diarrhea, and polyuria, followed by oliguria, anuria, hemoconcentration, azotemia, hyperphosphatemia, and uremic coma. Chronic administration of the hormone causes decalcification, bone cysts, deformities, spontaneous fractures, and metastatic calcification (page 630). Parathyroid hyperplasia and hyperparathyroid activity have been produced in animals by low calcium intake, vitamin D deficiency, renal damage, or continued injections of phosphate. (See discussions of renal rickets and hyperparathyroidism on pages 624 and 625.) Chronic hyperparathyroidism from hyperplasia or adenoma of the gland is termed *osteitis fibrosa* (von Recklinghausen's disease). It is characterized by pains in the back or extremities, disturbance of gait, fractures, deformities, decalcification, osteoclastomata, hypercalcemia, elevated serum magnesium concentration, increased plasma phosphatase activity, hypophosphatemia, metastatic calcification, renal calculi, polyuria, and negative calcium and phosphate balances. Renal impairment occurs frequently, and it leads to azotemia and hyperphosphatemia. Roentgenological examination reveals bone cysts, lacunar resorption, apposition, fibrosis of the marrow, and generalized osteoporosis. These skeletal changes are relatively late manifestations of the disease. Clinical hyperparathyroidism is treated by irradiation with x-rays, low calcium diets or partial extirpation of the gland, procedures which can readily produce tetany.

PANCREATIC HORMONES

Insulin is secreted by the β cells of the islands of Langerhans. The crystalline hormone is a protein; its molecular weight is 35,500, and its

isoelectric point is at pH 5.35. The international unit is equal to 48 γ of crystalline insulin or 40 γ of zinc insulin. Normal human blood contains approximately 0.02 international units of insulin per 100 ml. The chemistry of insulin, and its relations to carbohydrate metabolism are discussed on pages 304 to 312, and on page 317. Insulin stimulates appetite, gastric motility, glycogenesis, utilization of carbohydrate, fat storage, hypoglycemia, increased concentration of erythrocytic phosphate esters, lipopenia, and hypouricemia. The pancreatic endocrines, *diabetes mellitus* and *hyperinsulinism*, have been discussed on pages 339 and 334, respectively; and the therapeutic use of insulin, on page 342. A discussion of *lipocaic*, the lipotropic factor of the pancreas, may be found on page 219.

GASTRO-INTESTINAL HORMONES

Gastrin is a gastric secretagogue hormone produced by the pyloric mucosa (page 137). *Enterogastrone*, the chalone secreted by the intestinal mucosa, depresses gastric motility and secretion (page 138). *Secretin* is a basic proteose which is formed from prosecretin in the upper intestinal mucosa. This hormone and *pancreozymin* (from duodenal mucosa) stimulate the secretion of pancreatic juice; secretin has a smaller stimulatory effect upon the secretion of hepatic bile and succus entericus (page 142). *Enterocrinin* of the intestinal mucosa is a secretagogue for succus entericus (page 147). *Cholecystokinin* is a hormone of the intestinal mucosa, which stimulates contraction of the gallbladder (page 146). *Villikinin*, which stimulates motility of the intestinal villi, is also found in the duodenal mucosa (page 148).

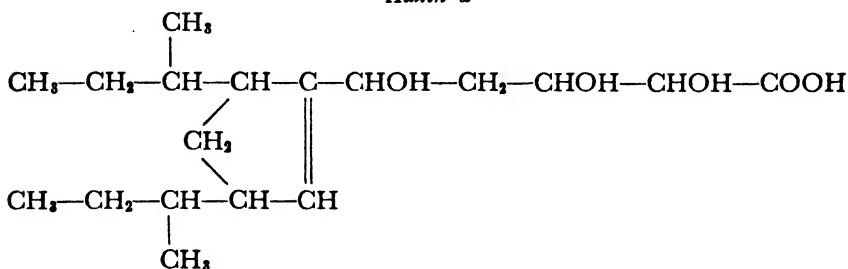
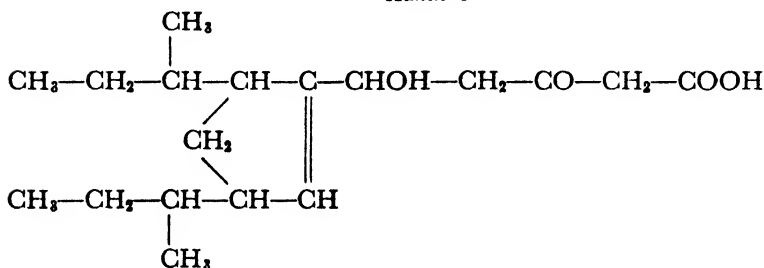
MISCELLANEOUS HORMONES

The pineal gland and the thymus have been classified as endocrine organs, but true endocrine functions of these glands have not been established with certainty. The pineal gland involutes before puberty. The thymus enlarges in such conditions as Graves' disease, Addison's disease, acromegaly, hypogonadism, myasthenia gravis, status thymicolymphaticus, and certain leukemic diseases; and it diminishes in size during wasting diseases. Thymectomy apparently affords symptomatic relief in some cases of myasthenia gravis. The gland normally begins to involute at the age of puberty, but it persists after early castration.

The physiological activities of *evocators* (hormones of differentiation) and of gene hormones have been discussed on page 496, and the chemistry and activities of the carotenoid conception and sex-determining factors (*gamones* and *termones*), on page 206.

The plant growth hormones, or *phytohormones*, include the auxins and traumatic acid; also the unidentified factors, caulocaline, rhizocaline, phyllocaline, and the flowering hormones, which stimulate growth of

stems, roots, leaves, and flowers, respectively. The auxins are cyclopentene derivatives.

Auxin a*Auxin b*

Auxin *a* is found in fungi, yeast, growing portions of plants, and in human urine. Auxin *b* has been obtained from malt and maize germ. Heteroauxin, or indole-3-acetic acid (page 378), is a derivative of tryptophane, which is found in small quantities in the urine, and is present in plants. The auxins stimulate cellular elongation, proliferation, nuclear division, and protoplasmic streaming in fungi and in higher plants. They increase cambial activity, root formation, and development of carpels and ovules. The auxins cause bud inhibition and delayed abscission, and are involved in the phenomena of epinasty, phototropism, and geotropism. Production of crown galls involves the formation of tumor cells and their growth stimulation by auxin. The growth-promoting effects of auxins are inhibited by iodoacetate. Heteroauxin, the homologous indole fatty acids, and related aromatic acids have been used experimentally to induce root and gall formation, epinasty, inhibition of lateral buds, development of flowers and fruit, and so forth. Acid derivatives of anthracene, fluorene, indole, naphthalene, and phenol tend to exert formative influences in plants, while halogen benzoic, halogen mandelic, halogen phenoxy, naphthoxy, and nitro benzoic acids can modify the patterns of plant organs. Acetylene, ethylene, and propylene, and low concentrations of carbon monoxide, produce anesthesia, cell elongation, proliferation, root formation, epinasty, and hyponasty. Traumatic acid, the wound hor-

none of plants, is a dicarboxylic fatty acid (page 210). This substance, and homologous acids, stimulate growth and cell multiplication in plants. The vitamins necessary for plant growth have been mentioned on page 665.

BIBLIOGRAPHY

HORMONES

General

- American Association for the Advancement of Science. The Chemistry and Physiology of Hormones. Lancaster, Science Press, 1944.
- American Medical Association. Glandular Physiology and Therapy. Chicago, 1942.
- CAMERON, A. T. Recent Advances in Endocrinology. Ed. 5. Philadelphia, Blakiston, 1945.
- KLEINHOLTZ, L. H. Hormones in crustacea. *Biol. Rev. Cambridge Phil. Soc.*, 17 : 91, 1942.
- SCHARRER, B. Endocrines in invertebrates. *Physiol. Rev.*, 21 : 383, 1941.
- (Series of authors.) The Relation of Hormones to Development. *Cold Spring Harbor Symp. Quant. Biol.*, Vol. 10, 1942.
- SELYE, H. Renotropic action of steride hormones. *J. Urol.*, 46 : 110, 1941.
- THOMPSON, K. W. Antihormones. *Physiol. Rev.*, 21 : 588, 1941.

Pituitary Hormones; Lactation

(See references to Pituitary Relations to Carbohydrate Metabolism, page 348.)

- CHOW, B. F. The purification and properties of certain protein hormones. *Adv. in Prot. Chem.*, 1 : 153, 1944.
- COLLIP, J. B. Corticotropic, thyrotropic and parathyrotropic factors. *J. A. M. A.*, 115 : 2073, 1940.
- ENGLE, E. T., and LEVIN, L. Gonadotropins. *J. A. M. A.*, 116 : 47, 1941.
- GEILING, E. M. K., and OLDHAM, F. K. The neurohypophysis. *J. A. M. A.*, 116 : 302, 1941.
- LONG, C. N. H. Growth and metabolic hormones of the anterior pituitary. *Ann. New York Acad. Sci.*, 43 : 383, 1943.
- PETERSEN, W. E. Lactation. *Physiol. Rev.*, 24 : 340, 1944.
- SMITH, P. E. Relationship of anterior lobe of the hypophysis to other endocrine glands. *J. A. M. A.*, 115 : 1991, 1940.
- SWANN, H. G. Pituitary-adrenocortical relationship. *Physiol. Rev.*, 20 : 493, 1940.
- VAN DYKE, H. B. The Physiology and Pharmacology of the Pituitary Body. Chicago, Univ. of Chicago Press, 1936-39. (2 vol.)
- WARING, H. The coordination of vertebrate melanophore responses. *Biol. Rev. Cambridge Phil. Soc.*, 17 : 120, 1942.
- WHITE, A. Lactogenic hormone and mammogen. *Ann. New York Acad. Sci.*, 43 : 341, 1943.
- ZONDEK, B., and SULMAN, F. Mechanism of action and metabolism of gonadotropic hormones. *Vitamins and Hormones*, 3 : 297, 1945

Estrogens and Progesterone; Menstruation; Pregnancy

(See references to Chemistry of Sterides, page 257.)

ALLEN, E. Physiology of the ovaries. *J. A. M. A.*, 116 : 405, 1941.ALLEN, E., *et al.* Sex and Internal Secretions. Ed. 2. Baltimore, Wm. Wood, 1939.BARTELMEZ, G. W. Menstruation. *J. A. M. A.*, 116 : 702, 1941.

BURROWS, H. Biological Action of Sex Hormones. New York, Macmillan, 1945.

CORNER, G. W. Corpus luteum hormone. *J. A. M. A.*, 116 : 591, 1941.

CORNER, G. W. The Hormones in Human Reproduction. Princeton, Princeton Univ. Press, 1942.

DOISY, E. A. The estrogenic substances. *J. A. M. A.*, 116 : 501, 1941.

REYNOLDS, S. R. M. Physiology of the Uterus. New York, Hoeber, 1939.

(Series of authors.) Sex Hormones. *Biol. Symposia*, Vol. IX, 1942.*Androgens*

(See references to Chemistry of Sterides, page 257.)

HOWARD, J. E. Chemical, physiological and clinical aspects of the androgens. *Bull. New York Acad. Med.*, 17 : 519, 1941.HUGGINS, C. The physiology of the prostate gland. *Physiol. Rev.*, 25 : 281, 1945.KOCH, F. C. The male sex hormones. *Physiol. Rev.*, 17 : 153, 1937.MOORE, C. R. Physiology of the testis. *J. A. M. A.*, 116 : 1638, 1941.*Adrenal Hormones*

(See references to Chemistry of Sterides, page 257.)

GOLDZIEHER, M. A. The Adrenal Glands in Health and Disease. Philadelphia, Davis, 1944.

INGLE, D. J. Problems relating to the adrenal cortex. *Endocrinol.*, 31 : 419, 1942.PARKES, A. S. The adrenal-gonad relationship. *Physiol. Rev.*, 25 : 203, 1945.SWINGLE, W. W., and REMINGTON, J. W. The role of the adrenal cortex in physiological processes. *Physiol. Rev.*, 24 : 89, 1944.*Neuro-effector Hormones*

(See references to Acetylcholine, page 259, and Adrenaline, page 499.)

NACHMANSOHN, D. Role of acetylcholine in the mechanism of nerve activity. *Vitamins and Hormones*, 3 : 337, 1945.*Thyroid Hormone*

(See references to Relations of Thyroid to Iodine Metabolism, page 637.)

MEANS, J. H. New approaches to the physiology of the thyroid. *Ann. Int. Med.*, 19 : 567, 1943.RASMUSSEN, H. Influence of the thyroid hormone on heart and circulation. *Acta Med. Scand.*, Suppl. 115, 1941.SOSKIN, S., and LEVINE, R. Recent advances in physiology of the thyroid and their clinical application. *Arch. Int. Med.*, 74 : 375, 1944.

Parathyroid Hormone

- ALBRIGHT, F. The parathyroids — physiology and therapeutics. *J. A. M. A.*, 117 : 527, 1941.
- POPE, A., and AUB, J. C. The parathyroid glands and parathormone. *New England J. Med.*, 230 : 698, 1944.

Pancreatic Hormones

(See references to Insulin, page 348, and Lipocaic, page 258.)

Pineal and Thymus Glands

- DAVIDOFF, L. M. The endocrinological aspects of tumors of the pineal gland. *Surgery*, 16 : 306, 1944.
- MCEACHERN, D. The thymus in relation to myasthenia gravis. *Medicine*, 22 : 1, 1943.
- MORGAN, E. A. The present status of the thymus gland in pediatric practice. *Canadian Med. Assoc. J.*, 44 : 41, 1941.

Gastro-Intestinal Hormones

(See references to Gastro-Intestinal Hormones, page 172.)

Phytohormones

- THIMANN, K. V. Auxins and the inhibition of plant growth. *Biol. Rev. Cambridge Phil. Soc.*, 14 : 314, 1939.
- THIMANN, K. V., and BONNER, J. Plant growth hormones. *Physiol. Rev.*, 18 : 524, 1938.
- WENT, F. W., and THIMANN, K. V. *Phytohormones*. New York, Macmillan, 1937.

ENDOCRINOSES

General

- American Medical Association. *Glandular Physiology and Therapy*. Chicago, 1942.
- GORDON, M. B. Pediatric endocrinoses. *Adv. in Pediatrics*, 1 : 229, 1942.
- GROSS, R. E. Neoplasms producing endocrine disturbances in childhood. *Am. J. Dis. Child.*, 59 : 579, 1940.
- LEMARQUAND, H. S., and TOZER, F. H. W. *Endocrine Disorders in Childhood and Adolescence*. London, Hodder and Stoughton, 1943.
- LOEB, L. The significance of hormones in the origin of cancer. *J. Nat. Cancer Inst.*, 1 : 169, 1940.
- LOEWENBERG, S. A. *Clinical Endocrinology*. Ed. 2. Philadelphia, Davis, 1941.
- SEVRINGHAUS, E. L. *Endocrine Therapy in General Practice*. Ed. 4. Chicago, Year Book Publishers, 1942.
- WERNER, A. A. *Endocrinology: Clinical Application and Treatment*. Ed. 2. Philadelphia, Lea and Febiger, 1942.

ZONDEK, H. *The Diseases of the Endocrine Glands*. Ed. 4. Baltimore, Williams and Wilkins, 1944.

Pituitary Diseases

(See references to Diabetes Insipidus, page 638.)

DAVIS, M. E., and HELLBAUM, A. A. Present status of gonadotropic therapy in gynecologic practice. *J. Clin. Endocrinol.*, 3 : 517, 1943.

SEVRINGHAUS, A. E. Dysfunctions of the anterior lobe of the pituitary gland. *J. A. M. A.*, 116 : 221, 1941.

SHEEHAN, H. L., and McLETCHE, N. G. B. Simmonds' disease due to post-partum necrosis of the anterior pituitary. *Brit. J. Obstetrics Gynecol.*, 50 : 27, 1943.

VAN DYKE, H. B. *The Physiology and Pharmacology of the Pituitary Body*. Chicago, Univ. of Chicago Press, 1936-39. (2 vol.)

Toxemias of Pregnancy

(See references to Toxemias of Pregnancy, page 502.)

Diseases of the Gonads

ALLEN, E., *et al.* *Sex and Internal Secretions*. Ed. 2. Baltimore, Wm. Wood 1939.

HAMBLIN, E. C. *Endocrinology of Woman*. Springfield, Thomas, 1945.

HAMILTON, J. B. Therapeutics of testicular dysfunction. *J. A. M. A.*, 116 : 1903, 1941.

HOFFMAN, J. *Female Endocrinology*. Philadelphia, Saunders, 1944.

MAZER, C., and ISRAEL, S. L. *Diagnosis and treatment of menstrual disorders and sterility*. New York, Hoeber, 1941.

NOVAK, E. *Gynecology and Female Endocrinology*. Ed. 2. Baltimore, Williams and Wilkins, 1944.

TWOMBLY, G. H. The relationship of hormones to testicular tumors. *Surgery*, 16 : 181, 1944.

WELLS, L. J. Descent of the testis: anatomical and hormonal considerations. *Surgery*, 14 : 436, 1943.

YOUNG, H. H. *Genital Abnormalities, Hermaphroditism and Related Adrenal Diseases*. Baltimore, Williams and Wilkins, 1937.

Diseases of the Adrenal Glands

(See references to Addison's Disease, page 638.)

GOLDZIEHER, M. A. *The Adrenal Glands in Health and Disease*. Philadelphia, Davis, 1944.

HARTMAN, F. A. Adrenal hormones in medical practice. *J. A. M. A.*, 117 : 1405, 1941.

KENYON, A. T. Adreno-cortical tumors—physiologic considerations. *Surgery*, 16 : 194, 1944.

KEPLER, E. J., and KEATING, F. R. Tumors of the adrenal cortex; diseases of the adrenal medulla. *Arch. Int. Med.*, 68 : 1010, 1941.

KEPLER, E. J., and WILSON, D. M. Addison's disease. *Arch. Int. Med.*, 68 : 979, 1941.

WINTERSTEINER, O. The adrenogenital syndrome. *J. A. M. A.*, 116 : 2679, 1941.

Cholinergic Diseases

(See references to Cholinergic Diseases, page 260.)

Diseases of the Thyroid

(See references to Thyroid Diseases, page 639.)

Diseases of the Parathyroid

(See references to Tetany and Hyperparathyroidism, page 638.)

Diabetes Mellitus and Hyperinsulinism

(See references on page 348.)

ADDENDUM

GUIDE TO BIOCHEMICAL LITERATURE

*"Men must be taught as if you taught them not,
And things unknown propos'd as things forgot."*

— ALEXANDER POPE.

The study of medicine is difficult, because the student must not only familiarize himself with numerous medical sciences, but he must also be able to apply his knowledge to the human body and its functions. Biochemistry has penetrated deeply into the various fields of medicine and surgery during the last few decades, and there is every indication that it is to become even more important in the future. The physician's skill in diagnosis and treatment depends to some extent upon his conception of fundamental metabolic principles; the medical student should strive to secure this foundation during the elementary course in biochemistry. It is of the utmost importance that he develop such familiarity with the subject that he can follow with understanding the future developments in medicine.

Within a few years after completion of his biochemistry course, the student will be confronted in the clinic with an amazing variety of pathological manifestations. He is then in urgent need of detailed biochemical information, which he must correlate with his clinical problems. It is the author's desire that this text assist the medical student in retracing his path, and in making speedy contact with particular phases of biochemistry. The subject matter has been arranged so that the presentation of each topic advances logically from pure chemistry, through metabolism and physiology, to pathology and medicine. Many of the specific data, which are required only occasionally, are arranged in tabular form for ready reference.

Years of intimate association with medical students and physicians have demonstrated to the writer that achievement in medicine and surgery depends upon diligent and constant study. The information which can be imparted in regular medical classes is merely a beginning. Those who succeed develop reading habits at an early date; only in this way is it possible to keep abreast with modern medical science. It is a constant source of enjoyment to the author to discover, in his fourth year conference sections, numerous students who have developed into active, responsible men and women, possessing reasonably extensive theoretical knowledge and a basic biochemical insight into medical problems. The ranks of the graduate physicians also contain their quota of earnest and productive scholars, who are genuinely interested in biochemistry. The encouragement and inspiration afforded by such associates have guided the author in his task, and he sincerely hopes that his text may be of assistance to those whose lives are devoted to the relief of human suffering.

The classified references listed in this text include many recent reviews on biochemical topics, but the student should be aware of three general sources of information, namely, general biochemical reference volumes, review journals, and abstract journals. The following are valuable reference volumes:

- BETHE, *et al.* Handbuch der normalen und pathologischen Physiologie. Berlin, J. Springer.
 DUNCAN, G. G., *et al.* Diseases of Metabolism. Philadelphia, Saunders, 1942.
 HAWK, P. B., and BERGEM, O. Practical Physiological Chemistry. Ed. 11. Philadelphia, Blakiston, 1937.
 OPPENHEIMER. Handbuch der Biochemie. Ed. 2. Jena, G. Fischer.
 PETERS, J. P., and VAN SLYKE, D. D. Quantitative Clinical Chemistry. Baltimore, Williams and Wilkins, 1931-1932.

One of the most suitable review journals for students is *Physiological Reviews*. Other important publications of a similar nature are:

- Advances in Enzymology*, New York, Interscience Publishers.
Advances in Carbohydrate Chemistry, New York, Academic Press.
Advances in Protein Chemistry, New York, Academic Press.
Annual Review of Biochemistry, Stanford University, Annual Reviews, Inc.
Annual Review of Physiology, Stanford University, Annual Reviews, Inc.
Biological Reviews of the Cambridge Philosophical Society.
Biological Symposia, Lancaster, Jaques Cattell Press.
Chemical Reviews.
Cold Spring Harbor Symposia on Quantitative Biology, Long Island, Long Island Biological Association.
Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie.
The Harvey Lectures, Lancaster, Science Press Printing Co.
Medicine
Vitamins and Hormones, New York, Academic Press.

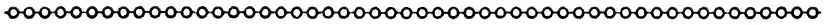
To investigate a given topic, the student should first read the latest review available, and subsequently he may obtain references to specific and original articles from the abstract journals, which furnish short summaries of individual contributions. The most inclusive of these journals, *Chemical Abstracts*, surveys articles of chemical interest in the fields of biochemistry, bacteriology, botany, medicine, nutrition, pathology, pharmacology, and physiology. It provides English abstracts of foreign articles in excellently indexed form. Other useful abstract journals are:

- Berichte der gesamte Physiologie und Pharmakologie*
Biological Abstracts
British Chemical and Physiological Abstracts
Nutrition Abstracts and Reviews

The last named journal contains complete sections on dietary therapeutics.

In the preparation of this text the author has consulted citations in *Chemical Abstracts* to January 1, 1946.

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